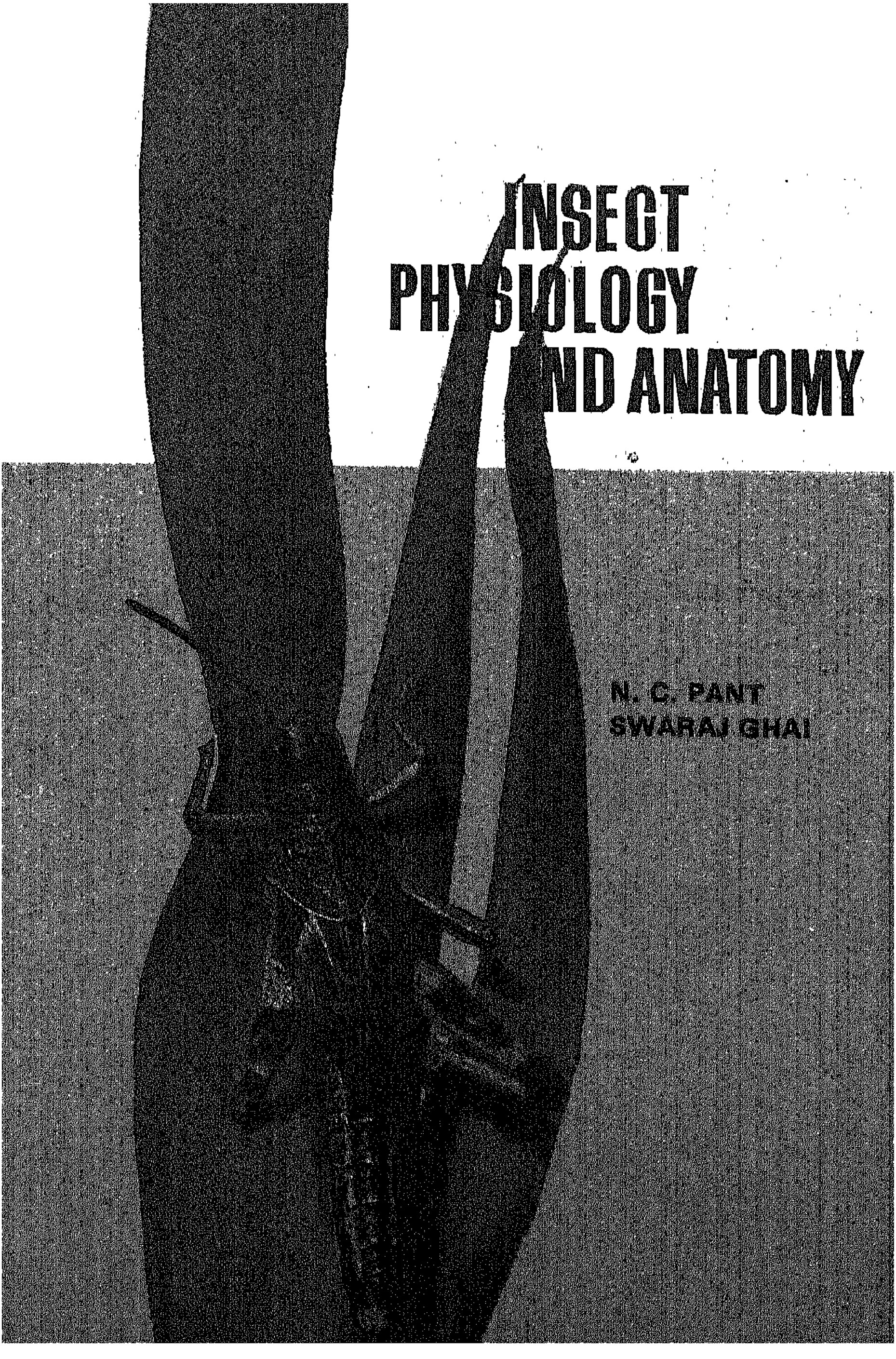
INSECT PHYSIOLOGY AND ANATOMY

N. C. PANT
SWARAJ GHAI



INSECT
Physiology and Anatomy
(Summer Institute Lectures)

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FOREWORD

Scientific knowledge is accumulating at such a fast rate today that a research worker working even in a very narrow field finds it extremely difficult to keep pace with the new knowledge and changing concepts. Opportunities for recurrent education have hence become absolutely essential for every one wishing to keep scientifically alive. The Summer School is a device which provides an opportunity for such continuous updating of knowledge. The Indian Council of Agricultural Research has therefore placed considerable emphasis on sponsoring Summer Institutes in different areas of agriculture, animal sciences and fisheries. While the concept of a Summer Institute is a good one, its effectiveness would largely depend upon the interest taken by the course Director and his associates on the one hand and the initiative and eagerness to learn, evinced by the participants. Judged by this yardstick, the Summer Institute on Insect Physiology held at the Indian Agricultural Research Institute in May/June, 1971 has been an outstanding success. There has been a comprehensive coverage of the important aspects of insect physiology and anatomy and it has been thoughtful of the different lecturers that they have taken the trouble of writing up their lectures in a form that would be useful to a large number of research scholars and students working in these areas. The present compilation would also provide an opportunity to the participants of the next Summer Institute in this area to assess the rapid changes which have taken place since 1971 in our understanding of the topics dealt within this book. It is appropriate that the book is dedicated to the memory of the late Dr. S. Pradhan whose contributions to the growth of entomological research and teaching have been monumental and who, above all, represented the spirit of integration of knowledge both in thinking and in action.

M.S. Swaminathan

PREFACE

THIS book is the outcome of compilation of lectures delivered at the "Summer Institute in Insect Physiology" sponsored by the Indian Council of Agricultural Research, New Delhi and held at the Division of Entomology, Indian Agricultural Research Institute, New Delhi from 12th May, 1971 to 10th June, 1971 with one of us (N.C.P.) as the Director of the Summer Institute. Participants from different parts of the country attended the Summer Institute and specialists from I.A.R.I. as well as from other institutions of the country delivered the lectures. All manuscripts received from lecturers have been included in the book.

Although efforts were made to select the topics of lectures in such a manner as to cover a wide spectrum of the subject of Insect Physiology, certain aspects could not be incorporated because of the short duration of the training programme. Further, unconventional topics like instrumentation, basic phenomena in living system, techniques *etc.*, were purposely included for the benefit of the students of Agricultural Entomology.

In the lectures that comprise this volume, certain amount of overlapping in the subject matter was unavoidable. The editors have tried to remove this as far as possible. Further, during editing, modifications in the manuscripts had to be made wherever necessary and any editorial error that may appear in the book are due to the oversight on the part of the editors for which they owe an apology to the authors who could not be contacted freely during the course of printing of the book due to various unavoidable reasons. Almost entire work of proof reading was done by the editors.

Grateful acknowledgement is made to the I.C.A.R. for providing funds, to our colleagues and organizers for helping in various ways in the publication of the book and to the authorities of the Indian Agricultural Research Institute for providing facilities to hold this training programme in Insect Physiology. Thanks are also due to the artists of the Division of Entomology, for preparing the diagrams.

This book is dedicated to the memory of late Dr. S. Pradhan, Head of the Division of Entomology, Indian Agricultural Research Institute, New Delhi.

N.C. PANT
SWARAJ GHAI

PREFACE TO SECOND EDITION

THE first edition of the book was published by the Division of Entomology, Indian Agricultural Research Institute, in 1973. It was so well received by the scientists and students, that all copies were sold out in less than two years. The book, based on the lectures delivered at the 'Summer Institute' did not contain information on certain important insect systems, resulting in, not as complete a publication as a book of this type should have been. This gap has now been filled in at least to a great extent, with the incorporation of five new chapters, on Origin of life, Insect integument, Insect wings and flight, Reproductive and Nervous systems.

It is hoped that the enlarged edition would meet the requirements of students better.

The editors are thankful to the Indian Council of Agricultural Research, New Delhi for providing funds for publication, and to Shri P. L. Jaiswal, Chief Editor (English) and Shri Krishan Kumar, Chief Production Officer, ICAR, for extending help in publishing the book. Thanks are also due to Shri A. K. Roy who prepared the illustrations for the new chapters and to Dr. P. D. Srivastava, Scientist S-3, I.A.R.I. for the continuous help rendered by him.

N.C. PANT
SWARAJ GHAI

Dedicated

to

the memory

of

LATE DR. S. PRADHAN

(1913—1973)

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(May 12, 1971—June 10, 1971)

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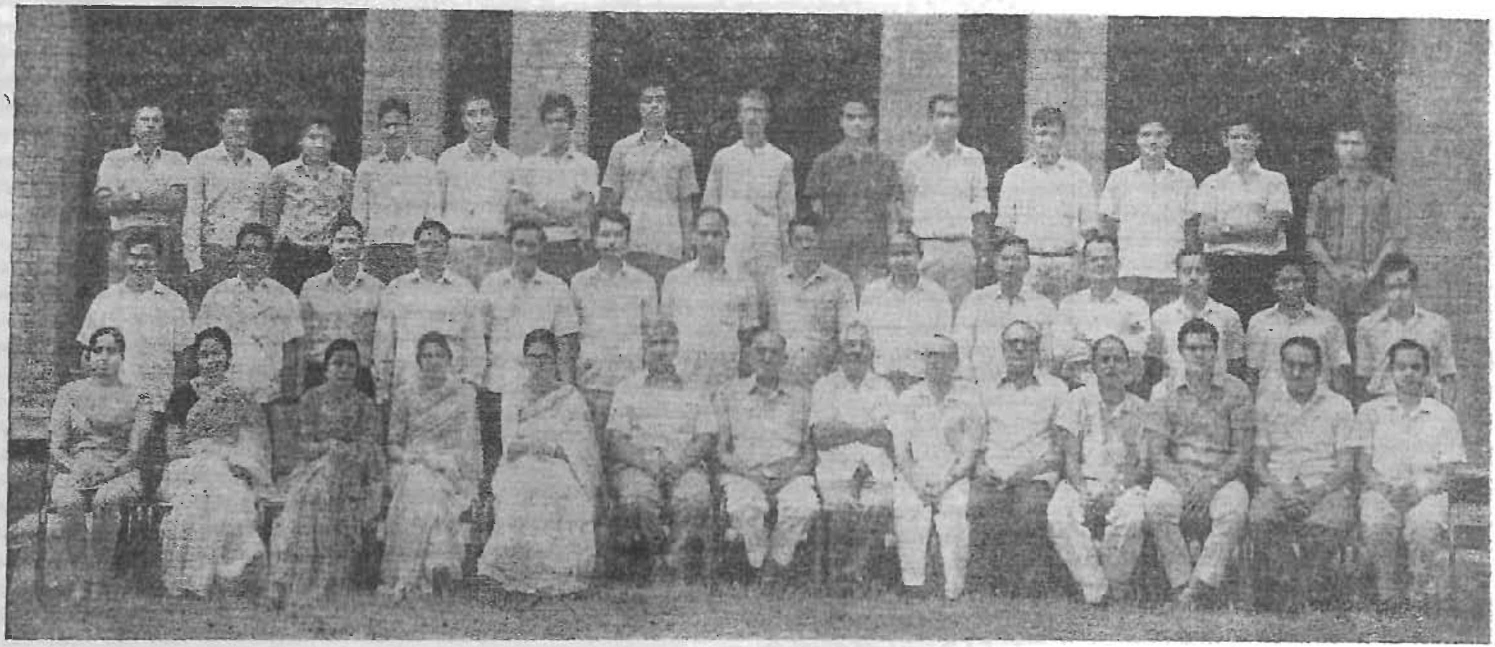

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1

ORIGIN OF LIFE

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What is life? A difficult but a right question to pose. It has not so far been answered satisfactorily. Biologists enumerate different characters on which matter can be grouped as 'living' and 'non-living'. But where to place a virus—under "living" or "non-living"? It is perhaps a connecting link between these two types of matter. Scientists now know that in early part of earth's history there was no life. Later, conditions became such that from "non-living" substances primordial life arose.

There are several hypotheses on the origin of life as it exists today. All these could be grouped into two schools of thoughts: (i) abiogenesis—which supports spontaneous generation of life from non-living materials and (ii) biogenesis which supports the idea that life arises only from the already existing form.

Abiogenesis. The world's great religions have supported this hypothesis. Earlier, philosophers of India and Greek believed that God created the life as it exists today. Aristotle thought that living beings can be generated out of non-living materials like decaying matter. Even as late as seventeenth century Jean Baptiste van Helmont, a Belgian scientist observed in an experiment that if a dirty shirt and wheat grains were kept in a box, mice were produced in 27 days. He, however, did not keep a control test by putting dirty shirt and wheat grains in a box which mice could not enter. Had he kept this controlled set, he would not have perhaps concluded that mice were spontaneously generated.

Biogenesis. Perhaps the first scientific investigation on the problem of origin of life was carried out by Redi, an Italian biologist during the mid-seventeenth century. He experimentally tried to prove that life is not generated spontaneously. He disproved the belief that fly maggots could generate spontaneously from rotting meat but demonstrated that adult flies were attracted to dead bodies and laid fertilized eggs to produce maggots. This and subsequent investigations by other workers, notably by Louis Pasteur gave a death blow to hypothesis of abiogenesis. The confidence of scientists believing in the abiogenesis origin of life was finally shaken to such a degree that they could no longer challenge the new thinking concerning life and its origin.

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The biogenesis has come to stay as an operating theory but it raises two questions. One, that if life begets life then all forms are the descendants of a common ancestor. If this is so, then why so many forms of organisms. Answer to this question lies in the theory of evolution which says that all living beings on the earth today are modified descendants of previous forms. The theory of evolution thus supports the theory of biogenesis and makes the latter more meaningful by the key words, "modified descendants". The second question is where and how first life came into existence? In other words, how primordial life originated on the earth?

Origin of primordial life on earth

Scientists have put forward several hypotheses to explain how life may have first originated on earth. According to some workers, life in the form of resistant spores of bacteria may have entered the earth with meteorites, from other parts of universe. This hypothesis may answer how life came on earth but fails to explain how it originated on the planet, where from it may have come. Further, it is not reasonable to presume that bacteria from outer space can tolerate and survive the rigours of extreme cold, heat and deadly radiations encountered during their journey to earth through space. The space research data collected with the help of artificial satellites have so far yielded no positive evidence of the presence of living matter in the space. It is likely that further researches may lead to a better understanding of the possibility of life travelling from one part of universe to another. Till then, the hypothesis that life came from outer space will have to be viewed with caution due to lack of evidence.

The widely acceptable hypothesis of origin of life at present is that life probably began 3000 million years ago from non-living chemicals and that the primordial living organism was a *heterotroph* (an organism which cannot make its food) and not an *autotroph* (which makes its own food *e.g.*, green plants). One may argue that this hypothesis then is the same as the early idea of spontaneous generation. There is an important difference. According to hypothesis of spontaneous generation, complex organisms can arise from non-living matter and that this is a continuous process which can occur any time. The heterotroph hypothesis maintains that non-living matter gave rise to unicellular, primordial life which during the course of million years of evolutionary changes, gradually modified to different types of plants and animals.

Lifeless era on the earth

Before life began, earth had passed through a stage when conditions were too hostile for any form of life. Earth came into existence about 4.5 to 5 billion (4500-5000 million) years ago out of a cloud of cosmic dust. The particles of the dust came closer to each other creating compression of matter generating tremendous heat. The temperature began to rise. This was followed by cooling over millions of years. The earth surface solidified forming earth's crust. The heavier metal sank to the centre core while lighter materials formed the crust. The gases escaping from reaction of various chemicals were held around the earth forming atmosphere.

The ancient atmosphere was a mixture of water vapours, ammonia and methane. There was no free oxygen, it was all tied up with water or in iron ore in granite (which contains 50 per cent oxygen). Earth's fund of nitrogen was similarly fixed in other compounds and there was little or no free nitrogen. Carbon was in the form of metal carbide, chiefly as iron carbide in rocks buried under the massive layer of granite and volcanic lava. All odds were against the creation of life but the gases and water vapours contained the basic ingredients of life *viz.*, oxygen and hydrogen (in water vapours), nitrogen (in ammonia) and carbon (in methane).

The lifeless earth was passing through dramatic events. Volcanoes were bursting all over, throwing internal matter over the earth's crust; ultra violet and cosmic radiations were bombarding land and sea. Water vapours in the atmosphere started condensing as earth cooled. Then came the rains which continued uninterrupted for thousands of years, filling the lower areas forming oceans of fresh water. The rivers, flowing through the rocks poured into seas, bringing with them innumerable types of dissolved chemicals from the earth's crust. Organic substances like amino acids and hydrocarbons were formed when atmospheric gases were subjected to cosmic rays and electric charges (lightning). It has been experimentally demonstrated that when a mixture of methane, ammonia, water vapour, hydrogen representing constituents of ancient atmosphere were subjected to an electric discharge, amino acids and other organic substances were formed. Such an event must have occurred in the ancient atmosphere also where gases were subjected to ultra violet rays and electrical discharge (lightning) resulting in the formation of amino acid and other matter. The organic substances thus formed were washed down to the seas which became the cradle of life.

The stage was now set for the creation of life in the sea water where temperatures were much lower than those on land. Sea water had necessary chemicals basic to life but a mechanism was still needed to hold the chemicals within a watery receptacle having reasonable stability of shape. Every living thing must have a body. Such bodies were provided by the physical forces acting on the interphase of organic matter suspended in water. Organic matter under certain circumstances can form jelly like droplets in water forming colloids. Water forms a very thin 'skin' around these colloids due to molecular cohesion. Through this peculiar skin of water, dissolved material can pass in and out. Such cluster of proteins, amino acids or other organic matters or their mixtures, which are held in small droplets within surrounding liquid are known as coacervates (Fig. 1-1). Coacervates have some kind of shape but they still are not a living matter. Their formation is one of the important steps in the hypothesis on the origin of life. One can make coacervates by mixing 5 ml of gelatin solution and 3 ml of the gum arabic solution in a test tube, adding acid drop by drop till the mixture shows cloudiness. Examination of a drop of this liquid under high power in a microscope would show small droplets of water of definite shapes with organic matter encased within them.

Formation of coacervates brought different chemicals together and eventually these may have reached to form more complex substances like proteins from a mixture of amino acids.Thus coacervates must have evolved someway of releasing the chemical-bond energy in amino acids and other organic molecules, and of making use of the released energy. This was a step forward toward the creation of life. The energy for activating chemical reaction was received from ultra violet radiation from the sun. Today ozone layer at the upper part of atmosphere absorbs the ultra violet rays but there was no ozone in the era of creation of life and hence these rays reached the earth unchecked. Besides the ultra violet, some of the chemicals—organic and inorganic, acted as catalysts which hastened the reaction at ordinary temperatures. Organic catalysts of proteinaceous nature were ultimately formed and constituted enzymes. Last step in life creation was the formation of enzymes which may have given complex coacervates a capacity to release and control chemically bond energy.

How coacervates or their modified descendants acquired the property of reproducing their own kind is not understood. This is an important missing link in the chain of events which led to creation of life from non-living chemicals. With the available data, interpretation and speculation one can only say that coacervates eventually may have developed into living organisms after struggling through millions of years.

It can be seen from the foregoing discussion that in spite of several theories put forward on the origin of life none could be considered flawless. A lot of information is yet to be gathered

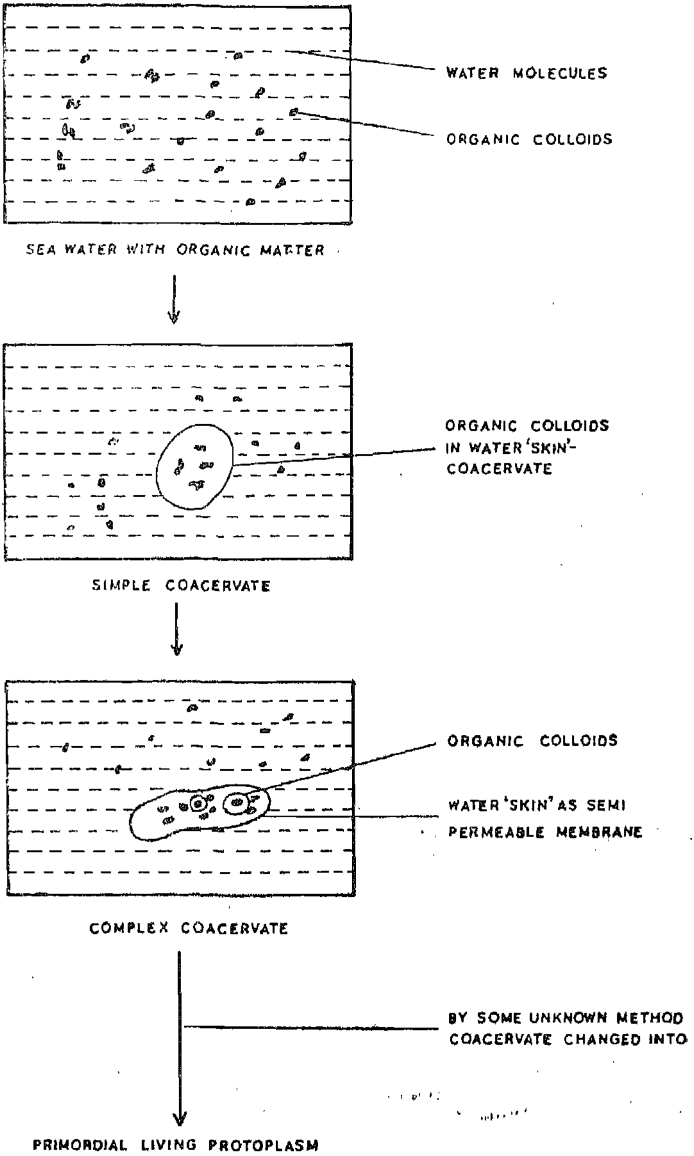
WATER MOLECULES
ORGANIC COLLOIDS
SEA WATER WITH ORGANIC MATTER
ORGANIC COLLOIDS IN WATER 'SKIN'- COACERVATE
SIMPLE COACERVATE
ORGANIC COLLOIDS
WATER 'SKIN' AS SEMI PERMEABLE MEMBRANE
COMPLEX COACERVATE
BY SOME UNKNOWN METHOD COACERVATE CHANGED INTO
PRIMORDIAL LIVING PROTOPLASM

Fig. 1-1. Formation of coacervates, the forerunners of life.

through pains taking investigations before the story of origin of life on the earth can be written fully supported by experimental evidences. The coacervate theory appears to be the most acceptable of all the theories propounded so far. One of the main objections of this theory had been that it presupposed formation of organic matter before the organisms came into existence. During the last five or six years it has been possible to show that an organic compounds are present elsewhere in the universe. Water, ammonia, HCl and formaldehyde have been identified in dust clouds outside our solar system. Remains of meteorites have shown to contain large amount of amino acids including glycine, analine, proline, valine and glutamic acid. These meteorites originated from earth and were thrown out during volcanic erruption into orbit. It is like that large pieces of rocks which went out into the space already contained traces of these amino acids and this was much before the life began on earth.

As the coacervates or any other forerunners of life started a chemical drama within the tiny droplets at a suitable spot on the seashore, chemical events, after long period of trial and error got so arranged as to perform functions of life. This was the beginning of physiology of organisms.

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2

NATURE AND SCOPE OF INSECT PHYSIOLOGY

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Physiology (Gr. *physis*=nature+*logos*=study) is that branch of biological science which is concerned with the function of living matter and its living processes at cell, tissue or 'organism as a whole' level.

Relation with other branches of science

In spite of its distinct existence as a subject, it is closely associated with morphology (science of structure at any level of organisation—cytology, histology or anatomy) on one hand and physics and chemistry on the other. Physiology is a science of life and therefore takes into consideration all those mechanics which give a matter the property of life. A mass of matter in the living state comprises of several chemical phenomena and is regulated by physical laws and that is where physiology is linked with biochemistry and biophysics. A physiologist or a team of biological scientists probing into the active life processes of living matter must apply principles and methods of physics and chemistry in order to explain reactions in terms of known physical and chemical forces. Such explanation often calls for an attempt to relate the life phenomena in terms of cause and effect. With the growth of physiology and its ever broadening scope and specialization, several branches now exist *e.g.*, cellular physiology, general physiology, comparative physiology, mammalian and human physiology, insect physiology *etc.* These branches deal in different animal groups or investigate into the underlying physiological principles with different aims.

Insect Physiology—a fundamental science

A general physiologist, for example, seeks to identify, interpret and apply the underlying unity or similarity of life processes in all living beings. In this effort to seek unity in diversity he often comes down to cellular level. On the other hand the aim of a comparative physiologist is to highlight the differences rather than the similarity of functions of different organs of animals or even individuals of a narrow taxonomic group. The Insect Physiologist is essentially a comparative physiologist because of the tremendous amount of diversity of functions in insects even at a very narrow taxonomic range.

Insect Physiology as a comparative study is of great importance for understanding the physiological and morphological adaptations which this class of animal has undergone to dominate our planet by its vast number of species and by its capacity to invade environments inaccessible or hostile to other forms of life. Attempts to generalise physiological processes of insects have often not met with success because of the endless specialization in the same type of organs in insects belonging to one order or family. The diversity of structure and details of functions of different parts of alimentary canal of a fly and mosquito (both belonging to order Diptera) bear a testimony to the above statement.

The subject matter of insect physiology is more experimental and fundamental in contrast to observational sciences like anatomy and morphology. Its scope is wide enough to seek an answer to the question how a certain phenomenon takes place and under what conditions? The approach of an insect physiologist is therefore designed to fulfil the basic inquisitiveness rather than the application of knowledge to solve practical problems—a sphere often owned by the applied scientist or technologist. It is probably this basic nature of the subject that insect physiology has been and still is a neglected branch of entomology in our country. This basic nature of the subject, though vitally essential, cannot always be motivated in terms of insect control (a requirement emphasized by most of the administrators) and hence the workers in this field have little to exhibit to a non-scientist that they are doing anything essential. In scientifically advanced countries both the Government and the public are fully aware that "Pure Science" is the soil where applied science develops and that if this soil is weak, technological advancement and its application will be blocked. We must therefore appreciate the importance of both fundamental and applied researches and realize that the scientific progress is impossible if any one of the two branches are neglected. If for every rupee spent on fundamental research, tangible saleable results are expected in return, the science and scientists will face the danger of immense harm. And when this happens the human welfare efforts will starve of the vital flow of new knowledge on which newer technology can be based.

The early history of insect physiology is indistinguishable from the history of biological science which received an impetus and opened a vast avenue for investigating mysteries of living form in 17th century when microscope was invented. Earlier to this scientists depended on observations based upon unaided eyes. The contributions made by Vesalius in 1543 on human anatomy and by Harvey in 1628 on blood circulation are considered as hall marks of the early progress of biology. Science of entomology started to develop from 1667 when Redi provided proof that maggots in meat are not spontaneously created but they develop out of the eggs laid by flies. In 1668 Malpighi worked out silkworm anatomy and in 1675 Swammerdam published an account of the internal anatomy of mayfly nymph.

Progress of work on the physiology of insects remained very slow although the knowledge in human and mammalian physiology later proved very useful in understanding the principles of insect physiology. The first book on insect physiology was published by Paul Marchal in 1911 followed by a textbook by Wigglesworth in 1939. It would not be wrong to regard Wigglesworth as a pioneer in the field of insect physiology.

A real impetus to the science of insect physiology was received soon after the discovery of DDT and of other new synthetic insecticides which posed several problems of resistance, specificity, phyto— and mammalian toxicity.

To solve these problems physiological approach was the only answer and thus a luxury science became a science of necessity. Due to the pollution of environment by the indiscriminate application of insecticides by farmers, housewives, and public health agencies, some countries have now banned the use of insecticides on foodstuff and are discouraging their use even on crop plants. A search for newer methods of control has now been intensified on a level never done before. The third generation methods of insect control like X-rays, radiomimatic compounds, pheromones and genetic manipulations are being explored for their immediate application in combating insect menace. The use of crop plant varieties resistant to insects is now being realised as a necessity and forms an integral part of several crop improvement projects in India. All these methods of control are based upon a thorough understanding of related physiological processes of insects. The use of resistant varieties as a method of control is difficult to achieve on a practical scale unless the bases of such reactions are understood. Similarly the use of pheromones and chemosterilants has physiological base on the understanding of which alone success can be assured.

Physiology—an applied science

The importance of insect physiology is amply clear from the above statement. The scope of the subject has been under constant change as more knowledge is gathered. With the discovery and subsequent large scale use of insecticides several problems of toxic hazard to men, livestock and other forms of life arose. Scientists in their efforts to discover safer pesticides eagerly sought answers to questions like how insecticides act and what makes insects resistant to pesticides? The answer to these and other questions lay in a realm where biology, chemistry and genetics meet. Modern insect physiologist unlike his older colleagues, often leaves the field of physiology and adopts principles and methods of biochemistry, physics, genetics and even ecology.

The *significance of insect physiology in economic entomology* can be best described in the words of Prof. V. B. Wigglesworth, "The physiology of insects is to some the handmaid of Economic Entomology. For although it is not the purpose of physiology to furnish directly the means of controlling insect pests, yet the rational application of measures of control—whether these be insecticides of one sort or another, or artificial interferences with the insect's environment—is often dependent upon a knowledge of the physiology of the insect in question. Physiology may thus serve to rationalize existing procedures or to discover the weak spots in the ecological armour of a species".

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BASIC PHENOMENA IN LIVING ORGANISMS

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ALL biological phenomena are chemical in nature. This might appear to be a sweeping statement at first but even such typically biological processes as the transmission of brain impulses to the nerves, or muscle contraction, are today known to be brought about by chemical reactions. The entomologists must be aware that even the phenomenon of behaviour (such as aggression, mating and feeding) in insects seems to be capable of being controlled, or even dictated, by chemicals. Thus we are led to accept, at least as a working hypothesis, that all reactions in living organisms, no matter how complex or how well organized, are nevertheless chemical reactions and therefore amenable, at least in theory, to study by chemical methods.

Then what is it that distinguishes a chemical reaction taking place in a biological system from one in a test tube? Of course, the most striking difference at first sight is that the biological reactions are very specific—in other words, only certain chemicals are involved, and they are handled and transformed by the organism to certain specific end products. But the most basic difference is that chemical reactions taking place in a living organism are almost invariably all catalyzed reactions. The phenomenon of catalysis exists in non-living systems also, but much less frequently and with much less 'specificity'. The "specificity" of biological reactions in a consequence of the properties of the catalysts involved, (which are invariably proteins called "enzymes") which determine what reactions can or cannot take place in the organism. It is clear, therefore, that the first step towards understanding of biological reactions is a study of the nature of "catalysis".

Only those reactions which can, according to the laws of thermodynamics, proceed spontaneously will be capable of being accelerated by catalysis. (As a rough guide, one might consider that a reaction which generates heat would be in this category). In the absence of a suitable catalyst, the reaction would frequently be so slow as to be imperceptible. An example would be the combination of nitrogen, oxygen and water to form nitric acid; the reaction does generate heat, but proceeds (in the absence of a catalyst) at an extremely slow rate. This is fortunate, as otherwise there would be no oxygen left in the atmosphere to sustain life as we know it. Another example is the oxidation of sugar to carbon dioxide and water. One gram-molecule of glucose, when oxidized completely would generate a considerable quantity of energy, 686,000 calories. Thus the reaction is thermodynamically feasible. However, one can keep glucose in solid form, or as a sterile solution in the absence of catalysts, for years and years without detectable change. One needs to add only a trivial quantity of the proper enzyme mixture (*e.g.*, cell-free extract from yeast cells) in order to bring about the oxidation of the glucose with consequent liberation of carbon dioxide, which can be detected within minutes. The enzymes have overcome or by-passed the *energy-barrier* present for the uncatalyzed oxidation of glucose. In other words, the *energy of activation* has been reduced by the catalyst. On the other hand, no catalyst is capable of changing the quantity of energy (or heat) produced or absorbed in a reaction, as this would be against basic thermodynamic principles. The consequence is that no catalyst can change the *equilibrium position* of a reaction, although the catalyst might tremendously increase the rate of approach to that equilibrium.

One of the characteristic features of the enzyme is its relatively great molecular weight. Also, each enzyme has in its molecular structure one or more specific sites for the attachment of substrate molecules, usually of much smaller weight than the enzyme. Thus it would be apparent that the rate of the overall reaction catalyzed would be proportional to the concentration of the proper complexes resulting from the attachment of the reactants (substrates) with the enzyme. The importance of this principle, first clearly enunciated by A. J. Brown in 1902, cannot be over-emphasized, since it forms the basis for all mathematical treatments of the velocity of enzyme-catalyzed reactions. Simple mathematical analysis, in fact, yields the results that (1) at very low substrate concentrations, the velocity of the reaction is proportional to the concentration of substrate and (2) at very high substrate concentrations the velocity of the reaction reaches a maximum and becomes independent of substrate concentration. These results were in fact observed by A. J. Brown during his studies on the enzyme-catalyzed hydrolysis of sucrose and it was this which led him to propose the formation of an enzyme-substrate complex as a necessary intermediate in the catalytic process.

This is in contrast to the "acid-catalyzed" hydrolysis of sucrose (studied by Wilhelmy in the middle of the nineteenth century) where the velocity of the reaction in aqueous solution is proportional to the concentration of sucrose over a wide range of concentrations.

A consequence of the large size of enzyme molecules is the possibility that they can each bind more than one small molecule. The importance of this would become obvious when it is realized that there is virtually no biological reaction wherein only a single reactant is involved. The picture we should visualize for an enzyme-catalyzed reaction is one of *simultaneous* and *proximate* binding of reactants. In the absence of the enzyme, the probability of one kind of molecule encountering another kind in free solution is very slender indeed. But when they are present side by side on the enzyme molecule, the binding to which at the same time positions them in the correct fashion so that the proper reacting groups can be brought together, the probability of reaction is enormously increased. In the language of thermodynamics, this is exactly equivalent to the statement that the enzyme has decreased the energy of activation. As an example of this kind of catalysis, one can consider the enzymatic oxidation of lactic acid to pyruvic acid. Here both the lactic acid and the oxidant which is a "co-enzyme" called NAD^+ (Nicotinamide adenine dinucleotide), are bound to the enzyme first, the lactic acid is oxidized (and the NAD^+ reduced to NADH) and then the products, *viz.*, pyruvic acid and NADH are released from the enzyme. The process of *simultaneous* and *proximate* binding of the reactants only partly explains the enormous increases in the rates of reactions brought about by enzymes. Other detailed theories and conjectures of the mechanism of enzyme action are however beyond the scope of this article.

There are known enzyme reactions where the velocity, instead of reaching a maximum with "saturating", concentrations of the substrate, decreases with higher concentrations. It is possible to explain such "substrate inhibition" by assuming that the substrate-enzyme attachment takes place by the enzyme binding to more than one point on the substrate : *e.g.*, an enzyme dealing with a substrate which we may designate as A-B, may have specific sites for A and B. In fact this assumption fits in nicely with our earlier supposition that the reacting substances are stereo-specifically oriented by the enzyme. There is also very strong independent evidence for such binding. When an excess of substrate is present, two molecules of the substrate A-B might bind to one of the enzymes, one through the A-group and the other through the B-group. Such a complex would be unproductive in the sense that it cannot be converted to products, and the results would be inhibition of the enzymatic reaction.

This type of inhibition is only one of many which serve to control the rate of enzyme-catalyzed reactions in the living organism. Some of the others are: (1) competition for the same enzyme by two or more structurally related substrates; this could fall in the category of what is termed as "competitive inhibition": inhibition of an enzyme reaction by the products of that reaction also belongs to this type, (2) the product of a later reaction in a sequence inhibiting one of the earlier reactions is termed as feed-back inhibition, (3) "non-competitive" inhibition, wherein a compound acts by combining with the enzyme at a site other than normally occupied by the substrate, and (4) reduction of the rate of a particular reaction due to the fact another enzyme is competing for the same substrate. The second variety mentioned here is usually of a type called "allosteric" by enzymologists and is not restricted to inhibition alone. Quite a few cases are known where an enzyme activity is *stimulated* in an "allosteric" fashion. In fact it is generally accepted that wherever one finds an allosteric inhibition phenomenon for an enzyme reaction, one should also find allosteric activators.

The foregoing discussion is intended to bring home the point that the secret of the precision with which enzymes act in controlling concentrations of cellular metabolites lies in the complex self-regulatory mechanisms to which enzyme systems are subject due to various inhibition and activation phenomena. Obviously, no such control mechanism exists in the case of pure chemical catalysis. One well-known instance of the operation of such mechanisms in biological systems is the Pasteur effect, which even after years of deligent study is not understood. The Pasteur effect is the finding that in many biological systems, the consumption of glucose is inhibited by the presence of oxygen. The purpose of this control is to economize on glucose: since metabolism of glucose in the absence of oxygen would stop with glycolysis, comparatively less energy is produced per molecule of glucose used up than in the presence of oxygen. In fact in the presence of oxygen very much less glucose is needed to produce the same amount of energy. If one looks into the reactions by which glucose is metabolized to produce energy, explanations would be forthcoming. The first step in the glycolytic pathway is the formation of sugar phosphates, a process needing inorganic orthophosphate. The form in which energy is produced and stored in this system is the compound called Adenosine triphosphate (ATP), the formation of which also requires orthophosphate. Thus, one of the many factors which bring about the Pasteur effect would be the competition for orthophosphate between the processes of formation of ATP and of sugar phosphates. In other words, since the availability of oxygen would facilitate the formation of more ATP, less orthophosphate would be available for the early steps of sugar phosphate formation. Thus, the concentration of ATP is kept under control. The above explanation is, however, far too simplified. It has been suggested, for instance, that the key step in the operation of the Pasteur effect is the enzyme, phosphofructokinase (PFK).

The important properties of PFK are that (1) it is inhibited by one of its substrates, ATP and (2) this inhibition is relieved by low concentrations of Adenosine diphosphate (ADP), Adenosine monophosphate (AMP) 3', 5'—cyclic AMP, inorganic orthophosphate and some other metabolites. These are, in fact, allosteric inhibition and activation processes; particularly, inorganic phosphate and AMP activate PFK in a synergistic fashion. The decrease in the ratio of ATP to other nucleotides orthophosphate, which would be caused due to lessened ATP synthesis under anaerobic conditions, would thus enhance PFK activity and the reverse would be observed under aerobic conditions. Thus the Pasteur effect is one of many physiological phenomena for which logical explanation can be found from a study of the properties of the biological catalysts, namely enzymes. The action of many hormones has also been explained due to their effect upon various enzymes.

It is to be clearly understood that a living cell is not a mere bag containing a cocktail of enzymes. Most of the enzymes are actually present attached to solid supports, such as cell-membrance, mitochondria, microsomes, *etc.* This property of structural organization must introduce a new and very important element in the regulation of metabolic reactions.

The discussion so far has been restricted to control of metabolic reactions by alterations in enzyme *activity*. Mention must be made of alterations in the *amounts* of enzymes present, brought about by various metabolites, through the processes of enzyme *induction* and *repression*. These phenomena are understood well only in micro-organisms, particularly bacteria, and knowledge in these areas is very meagre in the case of higher organisms.

The theme of this article has been that biological reactions are nevertheless, chemical reactions. However, due to the extreme complexities of the mechanisms of enzyme catalysis, ordinary chemical methods proved to be quite inadequate for the study of enzyme reactions and enzymologists had to invent mostly their own methods. The benefits to human knowledge have been enormous and the study of biocatalysis has in fact led to a better understanding and appreciation of the phenomenon of catalysis in general, as was predicted by the famous Swedish biochemist Hugo Theorell, very early in his career when he ventured into the realms of biochemistry.

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PHYSIOLOGICAL ADAPTATION AND SPECIATION

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INSECTS came on the scene of existence according to various estimates from 250 to 500 million years ago. The extent of antiquity of this origin of insects can be gauged from the fact that human being has come on the scene only one million years ago. Ever since such an early geological epoch insects have held their own against all odds and as a result of struggle for existence for such an astronomical length of time today the insects constitute the most dominant class of the animal kingdom. They also represent the culmination of evolutionary development in terrestrial arthropods.

DOMINANCE OF THE CLASS INSECTA

Insects constitute the largest class not only of the animal kingdom but of the living world as a whole including both the animal and the plant kingdom. This distinction of constituting the largest class is based on the consideration of the numerical strength of the known species of insects, the number of which is much more than that of all the known species of the animal kingdom put together. The estimates of both the absolute number of insect species and their relative proportion to all the known animal species vary within wide limits *i.e.,* from 700 thousand to about 1500 thousand species or from 70% to 90% of all the known species of animal kingdom. According to the estimate of Z. P. Metcalfe (1940) the knowledge about animals has been growing during the last two hundred years as follows: In 1758 when Linnaeus established binomial nomenclature he described only 312 genera and 4,203 species of animals of all classes. Out of this number 74 genera were of insects with 2,102 species. Later on in 1802 Sherborn listed all animals described between 1758 and 1800 and this list includes 3,204 genera and 58,833 species. Later since 1850 the annual publication of Zoological Records constitutes quite a reliable source of information. Counting the number of new species every 10 years published in these records it was estimated that a total of 948,780 species of insects were described between 1850 and 1940 and a total of 652,770 species of animals other than insects. This appears to be most reliable out of the available estimates although Z. P. Metcalfe has tried two other methods and the average of the three estimates has worked out to be 1,500,000 species of insects.

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* up to 5.2.1973

The various characteristic features which contribute to the insects' dominance as a class are summarised below:

A. Structural perfections

Exoskeleton: This is one of those characteristics which insects share with their all Arthropod allies, but insects seem to have fully exploited the mechanical advantage of their exoskeleton. The exoskeleton not only provides a much larger area for the attachment of muscles but also protects the muscles from mechanical injury. It affords excellent mechanism to protect insects against desiccation. But for the very effective water-proofing efficiency of insect's exoskeleton probably such minute animals as majority of the insects are, could not have evolved so successfully even as terrestrial forms much less as aerial ones. The main disadvantage of small size is comparatively much larger surface area per unit body weight which means greater vulnerability not only to mechanical injury but mainly to desiccation. The insects have successfully overcome both these difficulties due to their exoskeleton. Also, the exoskeleton has turned appendages into good tools for digging, preying, oviposition, *etc.* Lastly the exoskeleton maintains the shape of the body much more efficiently than the endoskeleton. Not only is old age not able to make its stamp on the form of many adult insects, but even death need not necessarily deface their beautiful shape. How far this advantage has made a difference in insects' success in the struggle for existence is difficult to assess correctly but it has certainly made the job of insect collectors much easier.

Small size: The evolutionary course followed by insects has led to the development of a large number of smaller individuals than a smaller number of large individuals. Undoubtedly this step immensely increases the chances of survival of the species. Not only larger the number larger the survival chances but easier it is for smaller individuals to sustain on small quantities of food available to individuals, and easier it is for smaller individuals to take refuge in small niches against inclemencies of weather as well as against adverse biotic environments. Also larger numbers resulting from smaller size increase the chances of variability and mutation thus making it possible for nature to conduct a larger number of trials in its evolution. Lastly smaller size leads to greater efficiency. It is one of the biokinetic principles that the contractile power of muscles depends on their cross-section, *i.e.*, area as square of a linear dimension, *i.e.*, a unit with two linear dimensions; the weight on the other hand depends on the cube, *i.e.,* on a linear dimension raised to the power 3, *i.e.,* on a unit with 3 linear dimensions. Hence when the size of an animal increases the power of its muscles increases with a lesser rate than its weight. Hence muscular strength is comparatively better in small animals. Thus it is no wonder that a flea with a length of 1/20″ can jump a horizontal length of 13″ and a height of 8″; if a man were to do similar feat he should jump 700 ft. and a high jump of 450 ft. This gives an idea of the relative efficiency of insect's small size.

Quicker speciation: As a result of exoskeleton and small size there has been quicker speciation. As already stated the number of species in the class Insecta is much larger than the total number of species of all other kinds of animals put together. Nobody seems to have ever tried to answer the question as to what the reasons are for this extraordinary amount of speciation in insects. After screening through the diverse characteristics of insects in general it has been concluded that the peculiar combination of the two main characters *viz.*, the possession of chitinous exoskeleton and the aerial mode of life has probably been the largest contributor to so much speciation in insects. Due to the mechanical rigidity of chitinous exoskeleton comparatively minute variations in the external genitalia obviously lead to reproductive isolation and consequent fixation of the species. This factor alone, however, cannot explain the large amount of speciation in insects because other classes of Arthropoda having exoskeleton do not have as many species. The development of the aerial mode of existence specially the acquisition of wings leading to life under much more diverse ecological habitats has provided the trigger for variations. Thus the extraordinarily large amount of speciation in insects is believed to have been brought about in the following manner: The chitinous exoskeleton made possible the evolution of minute insects with aerial mode of life. Then the life in diverse habitats resulting from the development of wings triggered a vast amount of variation; also the higher population of individuals resulting from the minuteness of the size increased the chances of variability and mutation; further quite a large number of those variations and mutations specially those associated even with slight alteration in the external genitalia got fixed as a large number of different species due to reproductive isolation resulting from the rigidity of the chitinous exoskeleton of the genital armature. This vast increase in the number of species immensely increased the economic potentialities of insects as a class.

Functional wings: This feature, the insects do not share with their other Arthropod allies and which gives insects a definite superiority. The power of flight greatly increased the statistical chances of survival and dispersal except on wind swept island, where reverse adaptation in losing the functional wings has taken place. It increased the feeding and breeding range and provided a new means of eluding enemies, of attacking a fast moving host (running or flying) and of finding a mate. Increased feeding range undoubtedly opened the way for the adoption of specific food specially in those areas in which the host or breeding medium occurred in small quantities and in scattered situations; *e.g.*, it would allow a species to adopt carrion as food since the individual with functional wings could seek out and reach carcasses which would not only be isolated but would also be suitable as food for only a short period. Also the same remark applies to mosquitoes, which, but for functional wings would not have been able to breed in small pools of water which dry in a short time. Not only have these functional wings enabled insects to search such scattered food and habitat as carrion and water pools but they have also enabled various species to undertake intercontinental migrations as in the case of locusts and butterflies. Some species have made this locomotion much more perfect by developing air sacs as in bees (for compensating for carrying honey) and flies (for compensating for the loss of one pair of wings). The structural perfection of these wings can also be gauged from the fact that a honey bee can fly at the rate of 5.7 miles per hour, a hover fly 7–8 miles, a hawk moth about 11 miles per hour and a butterfly even 57 miles per hour. This flight is brought about by means of wing beats in a regular succession the frequency of which has been estimated to be 250 per second in honey bee, 190 in hover fly, 85 in hawk moth and 12 in large butterflies.

Hexapod locomotion: Jointed nature of legs is a characteristics which insects share with their other Arthropod allies but the number of such legs has undergone an ideal stage of evolution in insects. It is instructive to appreciate that the six legs of insects on which the name *Hexapoda* is based represents the optimum number which no other group of animals has hit upon. The larger number of legs as in millipedes is bound to make the locomotion rather cumbersome but when the number of walking legs becomes less than six it is bound to create a problem of balancing during locomotion. For example, when a quadruped walks it has to balance its body on two legs when the other two are raised and moved forward. When a biped walks it has to balance its body on only one leg while the other moves forward. On the other hand if you carefully study the mode of insect locomotion on a solid surface you will find that the body always rests on a tripod while the other three legs move forward. Thus six is the smallest number for a stable equilibrium during all stages of terrestrial locomotion.

Compound eyes: Insects usually have a pair of compound eyes in nymphal and adult stages and in addition sometimes simple eyes too. Insects share this characteristic only with Crustaceans. These compound eyes are of very complicated structure which is not met with in other groups of animals. These eyes are called compound because each eye instead of having a single large cornea, consists of a number of hexagonal areas each representing the cornea of a discrete visual organ called the ommatidium all of which are compacted together. Thus ants have 50 to 400 facets or corneas in each eye, the housefly has 4000 in each eye, a swallow tail butterfly 17,000 while in certain sphinx moths and dragonflies this number goes beyond 50,000. It is obvious that the insect will not lose the power of vision completely if a few of their ommatidia are injured.

Scattered sense organs: Except the eyes none of the other sense organs are invariably concentrated on the head. The organs of hearing are sometimes associated with the antennae. Similarly the organs of taste and smell may occur on the antennae, mouth parts, or even on the tarsi or cerci. Such diffused and scattered nature of sense organs are bound to be advantageous to insects as this arrangement reduces the chances of all receiving injury at the same time.

Decentralized nervous system: The central nervous system is a ladder-like chain of ganglia along the ventral side of the body except one complex nerve centre in the head *i.e.*, the brain which is also dorsal to alimentary canal. This system, despite the highly evolved stage of insects as a class, is so decentralized that brainless insects can be artificially stimulated to walk, fly or even feed and, according to some, the living abdomen of the female silk worm moth when separated from the thorax can be fertilized by the male and stimulated to lay fertile eggs. How far this absence of centralization of the functions of the nervous system is useful in the preservation of the species in nature is obvious.

Direct respiration: This important physiological function is independent of the mediation of blood which in insects is not required to transmit the gases of respiration. These gases are taken to and from every minute part of the system directly through air tubes called trachea and tracheoles which have several openings called spiracle rather than a pair of nostrils.

Enteronephric excretion: The excretory function is performed by Malpighian tubules which open into the front part of hind intestine instead of opening directly to the exterior. Also the excretory products are semisolid rather than liquid urine. Thus it is a universal case of what has been described as enteronephric system of excretion which was considered at the time of its discovery in earthworms as a very unexpected phenomenon. Then people did not realize that the whole class Insecta has the same type of excretion. Obviously this system is also very well suited for water conservation as it makes the excretory function very much independent of water supply as water is not wasted in this function. There are further modifications in the same direction.

Thus despite the very minute size the whole of the internal organization of the insect system is highly complex. At the same time this is quite different from that of other groups of animals and is very admirably adapted for the success of this group of minute animal for life under a variety of conditions specially for arid existence.

B. Developmental characteristics

The development of at least a certain groups of insects is also unique in the animal kingdom. It is a specialization in which the full life history of the individual consists of four well-defined stages *viz.*, the egg, the larva, the pupa and the adult. Two of these stages, namely, the mainly feeding stage of the larva and the mainly reproductive stage of the adult are so different not only in structural details but also in their requirements of both food and habitat that their internal competition beween the parent and the off-spring for both food and shelter gets completely eliminated. Also the same individual is able to exploit at least two different kinds of food and habitat. Further, by the interpolation in between the larval and adult stages of a quiescent stage of pupa with great resistance and practically no requirement for food, the two active stages are best fitted into different seasons optimum for both the stages separately. Thus the development of metamorphosis has enabled the insects to adapt themselves to varied situations of existence much better than other animals, which do not possess this mechanism.

C. High fecundity and controlled reproduction

High fecundity as well as quick rate of development give insect prolific power of reproduction. It has been calculated that the progeny of a single moth can cover the entire dry surface of the earth to a depth of 80 feet within a period of one year in case all the progeny is allowed to live their normal life. This calculation is on the assumption that a moth lays just two hundred eggs and completes the life history in one month. Both these assumptions are quite normal for a large number of insect species while there are species of insects in which thousands of eggs are laid per female. Further, some species have increased their prolificity by having recourse to polyembryony while other species try to preserve their race even in the absence of males by resorting to parthenogenetic reproduction. While these are some of the attempts to preserve the species through increased fecundity there is also at the other extreme a completely controlled reproduction in some of the social Hymenoptera which have learnt to make their surroundings so safe as not to require a wasteful reproduction. In this connection it is also necessary to note that a number of social species of wasps, bees and termites have developed such a high degree of control of reproduction that all except one or a few females of the colony are rendered unfit for reproduction and constitute a class of sterile females. This strict division of labour between reproducing females known as queens and the labouring ones called workers represents the highest evolution of social order in the animal kingdom.

D. Specificity of food

Insects show great diversity of food habits, preferring one kind of food or other. A few species like locusts and army-worms have been able to overcome or to avoid interspecific competition by resorting to polyphagy so that they can feed on any kind of vegetable matter; yet another type like termites have adapted to feed on foods like cellulose for which there is little or no competition. Also, there is sometimes extreme degree of specificity not only to different plant species or varieties but also to different parts of the same plant (*e.g.*, root borers, stem borers, top borers of sugarcane).

E. Protective adaptations and devices

Protection against adverse biotic and climatic agencies is as much essential for the normal existence of the individuals as for the continuance of the species. Insects as a class have developed a variety of very ingenious ways for ensuring this protection. These methods have been grouped into several categories, *viz.*, (i) morphological adaptations, (ii) behaviouristic adaptations, (iii) construction of protective structures and (iv) selection of safety niches.

Morphological adaptations: The protective value of exoskeleton has already been discussed. This protective morphological basis has been supplemented in many cases by bristles, spines, hairs, scales *etc.* These in some cases afford more effective protection by their capacity to eject venomous liquid as in many caterpillars, blister beetles *etc.*

Often the minute size of insects affords protection against large predators. Again some insects are so large and of such grotesque appearance as might be quite frightening to their predators. Also insects as a class have developed high degree of protective colouration and adaptations used for camouflaging. These devices have been described under various categories like protective resemblance to surroundings in which they live (*e.g.*, stick insects, leaf butterfly, bark beetles, *etc.*,) and protective mimicry in which one species mimics some dangerous or distasteful species. Many butterflies and other insects which are themselves edible to birds, frogs, *etc.*, resemble in colour and shape, to other species which are poisonous or distasteful or resemble insects like wasps so that the mimic is able to dodge its predator. Then there is also, what is known as, warning colouration in which the venomous or distasteful insects are so prominently marked and coloured as to be easily distinguished from other species.

Behaviouristic adaptations: Some insects, for example stinging Hymenoptera take up such an offensive posture when threatened that the predator is frightened. Others adopt such dodging attitude as feigning death. Still others eject some kind of offensive liquid when the enemy approaches them (*e.g.*, caterpillars and lady bird beetles).

Construction of protective structures: To this category belong the termites or white ants. The galleries of white ants represent the air-conditioned pathways wherever even the workers have to go. There is no parallel of this kind in any group of animal including man. The hives of honey bee, wasp, the cocoons of pupae in so many species, the bag of clothes moth and other bag moths, the underground nests of ants, the nests of so many solitary wasps all come in this category.

Selection of safety niches: So many insects use their jumping or flying capacity to escape danger. Other search favourable niches for tiding over unfavourable conditions and sometimes it means long range migrations as in the case of so many butterflies.

ZENITH OF EVOLUTION

Finally the zenith of evolution depicted by such social insects as termites, ants and bees shows the degree of specialisation to which the insects have reached. They have evolved their own dairy, their own division of labour and social order and even their own language. All this evolution of all round efficiency is bound to be quite effective in their struggle for existence.

The foregoing paragraphs contain just very brief references to the structural, developmental, behaviouristic and organizational perfections of the insect world. They are meant to make the reader ponder over the problem and realize that in the interspecific wars of supremacy man faces a very tough challenge from the insect world. These minute creatures have held their own for hundreds of millions of years during which many gigantic forms have come and gone. Such being the case, it is no wonder that the control of insect pest is so difficult. Also the size of individual insects being small it is not surprising that the public finds it often difficult to comprehend the magnitude of the problem posed by these minute creatures. In fact this sense of complacency due to small size is itself an example how insects as a class exploit their minuteness for dodging humanity into a sort of false notion that after all, insects are too minute to pose any real danger. Generally insects are neither so minute as to be invisible to the naked eye and thus to create a kind of blind awe nor are they large enough to create any visual impression about the magnitude of their seriousness except under certain special circumstances like locust invasion. All this constitutes a part of the subtle game which insects have been playing for their survival and success in the interspecific struggle for existence and supremacy.

The most spectacular illustration or the extent to which insects have been dodging humanity is provided by the following history of revolution in pest control during recent decades which even scientists have not yet adequately grasped.

REVOLUTION IN PEST CONTROL

In agriculture, the production technology and the protection technology are so inextricably interwoven and interdependent that progress in the former is not possible without progress in the latter and the progress in the latter cannot be made use of unless there is progress in the former. This kind of inter-dependence is very clearly brought out by the history of the development of agriculture during the last few decades. In order to appreciate this phenomenon adequately the following line of rationale has to be properly grasped.

Recent studies on the extent of losses caused by insect pests to various high yielding varieties grown under high yielding crop husbandry practices have shown extraordinary high percentage of losses. For example, according to the trials carried out under All India Coordinated Rice Improvement Project, at 13 places in 1968 and 15 places during 1969, the all India average increase in the yield due to pest control in IR 8 was 37% with a maximum of 202% at Maruteru in 1968; these figures in 1969 rose to 108% as all India average in IR 8 and 194% in T(N)1 the maximum being 729% increase in yield due to pest control in IR 8 at Warangal and 1005% in T(N)1 at Maruteru. This shows the high degree of susceptibility of these varieties to insect pests and explains why it has not been possible to celebrate Rice Revolution so far while it has been possible to celebrate a revolution in wheat which is the safest crop from the field pests in India. *Similar data are available for a number of other crops.* Such experimental results in various crops coupled with frequently recurring pest epidemics in different parts of India during the last few years of the new strategy of agriculture, confirm two basic phenomena which have been well-known but not so spectacularly demonstrated earlier. These are: (a) that high yielding varieties are generally highly susceptible to pests unless they have been specially screened or synthesized for pest resistance and (b) that insect infestation increases considerably under high fertility status of the soil.

First revolution in pest control paved the way for and preceded the agricultural revolution by two decades. The first revolution in the control took place during the forties when the era of chemical control of pests was ushered in. Dr. Paul Muller got the Nobel Prize in 1948 for his discovery of the insecticidal properties of DDT which had set in motion the revolution in pest control technology during the mid-forties. This revolution in pest control technology preceded the agricultural revolution of the sixties by about two decades. During this period a large number of very potent pesticides became available and which enabled the breeders and agronomists to realize the potentialities of high yielding crop varieties. As these varieties are also very susceptible to pests under the conditions of high yielding crop husbandry, their potentialities could be realized only when highly effective pesticides were available to control their accentuated pest problems. It is with the help of a 'pesticide umbrella' that the breeder could pick up his high yielding genotypes (Fig. 4-1) which were earlier being eliminated and practically rogued out by pests to which they were much more susceptible; also the agronomist could observe the response of high doses of fertilizers which earlier used to get masked by higher pest infestation caused by the higher doses of fertilizer itself (vide response curves in Fig. 4-1). These two phenomena explain how the revolution in pest control technology paved the way for the agricultural revolution and formed the genesis of the *green revolution*. It is a fact of considerable scientific significance that Paul Muller, the discoverer of the insecticidal properties of DDT, who set in motion the revolution in pest control technology of the forties got Nobel Prize in 1948 *i.e.*, a little over two decades earlier than N. Borlaug who set in motion the agricultural revolution of the sixties and got the Nobel Prize in 1970.

Now a second revolution is needed: What, however, is still not adequately appreciated is that the production potentials which have been experimentally observed and visualised under the umbrella of pesticides can be realized on country-wide scale only under the same umbrella of pesticides (Fig. 4-2). At this stage a snag comes in. Under Indian conditions chemical control alone cannot take the country much further. It is quite feasible to apply the highly effective pesticides at the experimental stage and at the stage of national demonstrations. But when it comes to the stage of making large scale use of pesticides at the nation-wide scale the pesticide-use begins to have its own serious limitations. For India the cost of the pesticide needed per year works out on a very rough calculation basis, to about Rs. 700-800 crores. This constitutes a very huge input for Indian conditions when the national income from agricultural sources is roughly about Rs. 8000 crores and even if the nation somehow manages such a huge

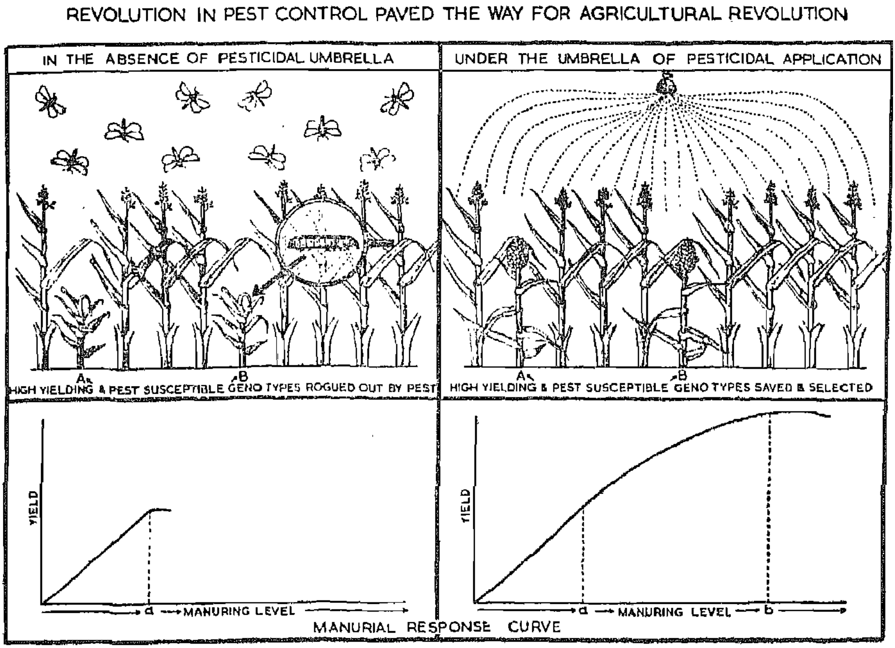
REVOLUTION IN PEST CONTROL PAVED THE WAY FOR AGRICULTURAL REVOLUTION
IN THE ABSENCE OF PESTICIDAL UMBRELLA
UNDER THE UMBRELLA OF PESTICIDAL APPLICATION
A
B
HIGH YIELDING & PEST SUSCEPTIBLE GENO TYPES ROGUED OUT BY PEST
A
B
HIGH YIELDING & PEST SUSCEPTIBLE GENO TYPES SAVED & SELECTED
YIELD
a → MANURING LEVEL
YIELD
a → MANURING LEVEL → b
MANURIAL RESPONSE CURVE

Fig. 4-1. Revolution in pest control paved the way for agricultural revolution

input in the form of pesticides, the adverse side effects of such a vast scale use of pesticidal chemical will go out of control; these are health hazards, environmental pollution, the evolution of resistant pests and an upsetting in the balance of nature. 'Pesticidal Umbrella' is however not feasible on nation wide scale. Another revolution in pest control technology is therefore needed through very high research investments. Matching of pest control effort with production goals has to be viewed against this background.

Under the circumstances given above it is quite clear that another revolution in pest control technology is needed for taking the present green revolution to its next logical stage. This second revolution is to take place in the form of what has begun to be called 'Directed Integrated Control' which has to be carried out under the direction of pest control practitioners and in which all the feasible non-chemical control methods have also to be properly integrated. This revolution has to be brought about step by step. To start with a highly adequate surveillance of the quickly changing pattern of pest infestation has to be carried out by a highly competent team of entomologists with adequate ecological background. This has to be

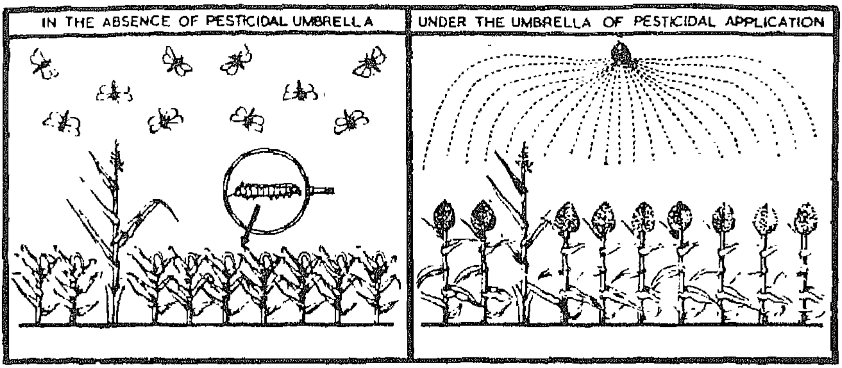
IN THE ABSENCE OF PESTICIDAL UMBRELLA
UNDER THE UMBRELLA OF PESTICIDAL APPLICATION

Fig. 4-2. Fate of high yielding pest susceptible genotypes grown in the absence and presence of "pesticidal umbralla".

a research-cum-control activity aimed firstly at exploring the various intricate ways in which the agricultural revolution is bringing about a revolution in pest infestation intensity and secondly to get those infestations nipped in the bud. Of late the word surveillance has been picked up and is being exploited in some quarters but the science and philosophy of the same do not seem to have been properly grasped.

It is well-known that man is a slave of circumstances. So are all other living organisms too. But man often, not knowing what he knows not, interferes with circumstance and environment in such a way that he becomes a slave of his own creations. This is what is happening in Indian agriculture. This is what happened in Nicaragua. It has been reported that during the early sixties the chemical control of cotton pests in Nicaragua created, as expected, a very spectacular effect and the crops thus protected gave the highest yield per hectare in the world. The people felt that they had got the panacea. Chemical control firms received huge orders and they enthusiastically executed the same. The result, as reported, was that the pests became resistant and the balance of nature got upset and by the late sixties it became practically impossible to grow cotton where its yield was highest in the world during the early sixties. We should learn a lesson from such gigantic human follies. We must keep very competent and sensitive fingers on the pulse of nature and also continue to feel how nature is reacting to our interferences. There must be an ecological approach in planning crop sequences particularly from the pest and disease point of view.

It is often difficult to convince people when it is stated that even very slight changes in crop ecology can bring about a pest epidemic. Hence a numerical illustration is necessary.

Suppose an insect lays 100 eggs and its population is constant over a period of a few generations as it often happens. This means that only two of the 100 eggs reach the adult stage in each generations *i.e.*, there is 98% pre-imaginal mortality. If the number of eggs laid per individual is 1000 then the pre-imaginal mortality will work out to 99.8%. If this pre-imiginal mortality is somehow reduced by 2% in the first case or by 0.2% in the second case, the population will double itself in one generation of, say two to three weeks and an epidemic can result within two or three generations *i.e.*, within two or three months. Now it should not be difficult to visualise a reduction in mortality by 0.2% as a result of changes in crop ecology leading to pest epidemics.

For bringing about this second revolution in India quite a sizeable research investment is needed and research and administrative efforts should be earnestly directed to this end in view.

5

PHYSIOLOGICAL APPROACH IN INSECT TAXONOMY

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TAXONOMY and nomenclature together form the Code Language of any biologist. When the name of a taxon is uttered, it gives a good deal of understanding, more than any ordinary word in any language, perhaps more than the amount of matter that can be accommodated in a hydrolically compressed bale. It conveys ideas of an infinite number of attributes of the constituent members of that taxon pertaining to all other fields of the biological sciences, such as morphological, physiological, ecological, ethological, distributional, genetical, toxicological, parasitological and so on.

The words 'taxonomy', 'systematics', and 'classification' have been used alternatively for the same subject by various biologists. However, Simpson (1965) has attempted to segregate these, giving the following definitions for each. Thus, according to him, in the zoological science, 'classification is the ordering of animals into groups (or sets) on the basis of their relationships, that is associations of contiguity, similarity, or both': 'systematics is the scientific study of the kinds and diversity of organisms and of any and all relationships among them' and 'taxonomy is the theoretical study of classification, including its bases, principles, procedures, and rules'. Nevertheless, in actual practice it is rather difficult to completely dissociate each of these under discrete compartments. Nomenclature deals with the naming of the groups under which the various animals are segregated in accordance with any of the above terms.

From the early days of human civilization attempts have been in progress to segregate the various animals under distinct categories. It is perhaps from such attempts that even a part of the script of any language too may have been evolved, as seems evident from the ancient Egyptian hieroglyphics. Wilson and Donner (1937) have presented the various systems of animal classifications, specially with reference to insects, that have been followed by different biological philosophers in the past. These include the 'habitat or locality system' of Agricola and others, the 'alary or wing system' of Muffet and others, the 'metamorphotic or transformation system' of Swammerdam and others, the 'ciberian or maxillary system' of Fabricius and others, the 'circular system' of Macleay and others, the 'embryological system' of Home and others, the 'philosophical system' of Oken, the 'cephalization system' of Dana, the 'pupal system' of Swammerdam and others, the 'thoracic system' of Schoch and others, the 'segmentation system' of Prell and the modern 'evolutionary system'. In very recent years a new approach ignoring the evolutionary hierarchy, known as 'numerical taxonomy' or 'taximetrics' has sprung up.

The classification presently followed by most biologists, however, is based on the Linnaean hierarchial system established in the tenth edition of his 'Systema Naturae' (1758). This takes into account the natural relationships of various living organisms on the basis of homology and analogy. The investigations on comparative morphology by Cuvier and subsequent workers led to evolutionary thoughts and the complete overthrow of the biblical ideas of a 'Special Creation' of all life by an Almighty God. This has been substantiated by evidence drawn through the efforts of Lamarck (1809) and Darwin (1859). With the establishment and acceptance of the ideas of 'Organic Evolution' attempts at biological classification on the basis of the evolutionary hierarchy began to be appreciated as a mere natural system. Remnants of past life taken as fossils began to be looked upon with deep interest. Comparative investigations on these with the living forms led to the concepts of evolutionary trees. Yet, how evolution is practically at work in nature remained an enigma. Attempts to probe into the principles of the varying character inheritance from generation to generation paved the way to the modern field of genetics.

Mendel's enunciation of the primary laws of character inheritance (1866), the 'Mutation theory' of De Vries (1909), Morgan's 'theory of the gene' (1930), 'One gene-one enzyme' idea developed by Beadle and Tatum (1941), the establishment of Mischer's deoxyribonucleic acid as the veritable stuff of life by Avery and his co-workers (1944), Crick and Waston's exposition of the structure of DNA as a double helix of polynucleotide chains held together by hydrogen bonding between four bases, thymine, adenine, cytosine and guanine, Pauling and Sanger's finding about proteins as chains of amino acid bases, and the results of the very recent researches of Nirenberg, Khorana, and Holly regarding the chemical pathways of protein synthesis, all have contributed to an understanding of the biological evolution as nothing but a continuation of the chemical evolution.

The process of evolution may then be briefly summed up as follows. Perhaps it may be necessary to start with the formation of elementary matter itself. With the concepts of photons and the quantum theory, it is getting increasingly difficult to dissociate energy from matter. Of what may be considered as matter, the common building blocks are the fundamental particles, protons, electrons and neutrons. The combinations of these in ascending numbers built up the entire atomic series starting with Hydrogen to the higher radioactive elements. The valencic properties of these elements paved the way to the molecular series of chemicals. The elements of primary importance with reference to biological evolution are hydrogen, oxygen, carbon and nitrogen. These gave rise to the essential biological molecules, water (H_2O), carbon dioxide (CO_2), ammonia (NH_3), methane (CH_4), *etc.*, in differential molecular combinations. Further progressive chemical evolution from these built up the macromolecules of sugars, fats, amino acids, proteins, purines, pyramidines, *etc.*, till the stage of nucleotides and nucleic acids were reached. The attainment of the latter may be considered as the starting point of biological evolution. The breakdown of these macromolecules and re-assembly to form others, forms the basis of all life phenomena. These steps are fairly well indicated by Paul Weisz (The Science of Biology, 2nd Ed., 1963).

Through the physiological investigations carried out so far, it is now well known how proteins synthesized by the different cells of an organism, acting as enzymes, hormones, *etc.*, ultimately lead to its phenotypic expression. Biochemical researches have also shown the pathways of differential protein synthesis through the action of differentiated DNA molecules. It is now clear that a slight DNA change should cause the synthesis of quite a different protein which works quite in a different way towards the external expression of a full fledged organism. While it has been the phenotypic expressions of organisms that have been so far taken into account in taxonomy, basic factor in biological speciation being a DNA transformation, taxonomists must now take this aspect into full account. Thus from the concepts so far in vogue, which are purely that of a 'phenotypic evolution' one can readily conceive of a 'polycellular evolution' within a multicellular organism, that is basically responsible for the organic evolution at work. DNA transformations thus form the crux of the problem in evolutionary taxonomy.

Just as the mere possession of a gun does not permit it to be fired off without being loaded with a bullet and then the necessary pressure applied to pull its trigger, so also the mere presence of a DNA within the cellular system cannot work without the availability of the necessary chemicals and the required energy source. While a good deal of knowledge has recently been accrued on the chemical aspects of the phenomena of cellular growth and reproduction, very little knowledge has been gained on their physical aspects. Thus biophysics still remains a much younger baby than biochemistry. It may be noted that while the availability of the necessary chemicals towards the formation of daughter cells from a single dividing cell remains equal, any differentiation that may be accomplished between them has to be attributed to the differential physical factors that may be acting from the two sides of the line of cleavage. Thus physical aspects may even be more important towards an understanding of polycellular evolution. In fact, both physics and chemistry may have to be invoked simultaneously. Polycellular evolution being the basis for polyphenetic evolution, the same approaches are to be made towards these investigations as well.

The basic physical fact that two bodies cannot occupy the same space at the same time, leads to the corollary that each body must work in a different physico-chemical niche, whether it be a DNA molecule within the nucleus, the actively dividing cells of various organs, or be it entire organism in the process of evolution. Investigations in cellular biology have brought out facts regarding the source of the necessary chemicals from the blood serum and the required energy from ATP-ADP transformations, for any cell to be at work. From the investigations of Muller onwards to the present day, how phenotypic changes can be experimentally brought about in any organism through chemical or physical alternations in its environment have also been demonstrated. How these changes are actually brought to bear on the various organisms towards a natural evolution at work remains yet far from conclusive.

As one attempts to take a rough census of the so far recorded biological species of the world, one finds that as against about a hundred thousand species of higher plants, well over one and a half million species of insects alone are there. Insects, of course, form 75-80% of the animal kingdom. Assessing from the above estimates and taking into full account all forms of plant and animal life, there is an appallingly high preponderance of the world fauna over the flora. The motility of the animals, geographical isolation thereby readily caused selection pressure in the different ecological niches they have sauntered to, have all been considered as causative of their higher amount of speciation. These, however, do not fully explain the basic mechanism of DNA transformations that are ultimately responsible for speciation.

Even a cursory observation of the different taxa of animals would show a certain amount of correlation between the mass and size of the constituent members of any taxon and the amount of speciation attained therein during the course of evolution. Thus on a rough estimate the massive and bulky chordate animals have about fifty thousand species whereas the much lighter and smaller insects comprise over a million and a half species as already indicated. The Arthropoda forms the largest phylum and the Mollusca second to that in number of species and these are all comparatively much lighter and smaller than the mammals and other vertebrates. It is a common observation that most of the lighter and smaller animals are bodily lifted up by high velocity winds and kept buoyant with the upper air currents. During this period they are likely to be acted upon by cosmic radiations, ultra-violet rays, electrostatic fields between clouds, *etc.*, of the higher atmosphere. The effects of such physical factors in inducing genetic mutations are too well-known through the efforts of Muller and a larger number of subsequent workers. Therefore it is possible to conceive that during the period of their passive flotation along with the upper air currents, the meiotically dividing cells of their gonads may be subjected to a DNA transformation. As the strength of the air currents abates, such organisms come back to the ground either actively or by the passive gravitational pull, settle down, and start breeding giving rise to a generation of differentiated phenotypic forms. In accordance with the survival value of such phenotypic mutants in the various ecological niches where they settle, further apparent evolutionary effects are attained.

Here, a distinct difference between the members of the two kingdoms are evident. While entire animals with their gonads comprising of cells potentially capable of meiotic divisions for the production of sexual gametes, are lifted up by winds, of plants only small twigs, leaves, lighter or winged fruits and seeds, spores, *etc.*, are so raised; since no meiotically dividing cells are involved in such plant parts, any DNA transformation effected in their cells are not likely to make any impact on evolutionary phenotypic speciation. Even when these parts come down they have very little of survival value either. Perhaps this is borne out by the lesser amount of speciation presented by the plant kingdom.

It must be pointed out that the physical conditions of the upper atmosphere cannot be exclusive for the production of gene mutations. Chemical and physical factors of the ground or water collections too can cause a certain amount of speciation. This is because no two ecological niches can be identically .the same as already pointed out. However, from the effects of radiations and electrical states known now to have the more profound influence in chemical transformations, it is possible to conceive that the maximum amount of biological speciation is possible in the upper atmosphere where these factors are definitely more pronounced.

The foregoing account brings out clearly that the first steps in biological speciation start with a purely physico-chemical *i.e.*, a physiological basis and continues on with geographical isolation, selection pressure, *etc.*, in the phenotypic forms that are obtained. That phenotypic forms do not truly *depict specific differences* between various organisms too has now become an established fact. To cite a single example, it is too well known now that human beings have developed distinctive non-compatible blood groups without much of phenotypic differentiation, so much so that the modern definition of a species as 'an evolved or evolving, genetically distinctive, reproductively isolated, natural population, may not hold true to the humanity as a whole, and strict application of this definition may bring *Homo sapiens* just a heterogenous mixture of species. Physiological approaches then take a leading role in biological taxonomy.

Several physiological approaches have been made within very recent years in biological taxonomy. Most of these have been with reference to the plant kingdom or the larger vertebrates of the animal knigdom, wherein the morpho-taxonomic ideas are more firmly established. Coming on to the invertebrate animals, particularly with reference to insects despite their astronomic amount of speciation chemotaxonomic investigations so far conducted are too negligible. This may be because of the fact that among these even the morpho-taxonomic limits have yet to be ascertained. It is no wonder then that in some of the recent books dealing with such an approach, for example 'Biochemical Systematics' by Alston and Turner (1963) and 'Chemotaxonomy and Serotaxonomy' edited by Hawkes (1968), hardly any reference to the physiological taxonomy of Insecta is covered. Some chemotaxonomical work has been accomplished, however, by some pioneering entomologists in this field.

Chemotaxonomical approaches towards insect studies are feasible along three lines, *viz.*, (1) serological, (2) histochemical, and (3) the chemistry of the excreted products of insects. Serological investigations again may be resolved into immunological, chromatographic, or electrophoretic, though all these pertain to the chemical composition of the serum of the blood or haemolymph which carries the necessary chemicals to feed the cells in development and reproduction.

The immunological approach is based on the preciptin reaction discovered by Kraus (1897), applied first by Nattal (1901) to broad taxonomic problems, and further elaborated with refined techniques by Boyden (1943). This concerns with the nature and interactions of antigens and antibodies. Antigens are substances which cause formation of antibodies when introduced into the blood. Antibodies obtained from immunized animals from their blood sera, through antigen inoculations from a foreign source, are serum globulins which precipitate out with suitable techniques. The preciptin reaction consists in the formation of a visible precipitate at the interface when one brings together an antigen with the corresponding antisera. This approach has been resorted to towards a study of the Orthoptera by Leone (1947), Pauly (1955) and Downe (1961), in the Hymenoptera by West *et al.*, (1959) and Lawlor (1949), in the Lepidoptera by Martin and Cotner (1934) and Brown and Heffron (1928), in the Diptera by Cumley (1940), Fox (1948), Downe (1963) and Simon (1969) and in the Coleoptera by Butler and Leone (1967) and Basefort *et. al.*, (1968). Antisera has been obtained by these workers by introducing either homogenized extracts of whole ground-up insects or only the sera of the insect blood into healthy rabbits or chickens. Comparative assay for the taxonomic interpretation has been made through different techniques, such as photodensitometric techniques, micro-immunoelectrophoresis, double-diffusion test, *etc.* The various papers by the above authors may be consulted for the detailed procedures adopted by each. The exact methodology followed by these workers varies slighty and standardized techniques to deal with insects have yet to be evolved.

Chromatography is a technique used to separate complex mixtures into their components. In column chromatography a solution containing the mixture to be analysed is poured into a "column"—glass tube packed with absorbing materials. Clear solvent is then poured down the column. Each component in the mixture has a characteristic affinity for the solvent and for the absorbent; hence it will occupy a definite position in the column. For example, substances most strongly attracted to the absorbent and most weakly held in solution will adhere to the topmost part of the column. The separated substances will form a succession of distinct coloured bands; hence the name "chromatography" (analysis by colour). The separated components are extracted and analysed. In paper chromatography filter paper is used instead of absorbing material. Paper chromatography as a tool in insect taxonomy has been made use of by Micks (1954, 1956).

Electrophoresis is another technique used to separate mixtures into their components. It is based on the fact that the components carry electric charges of varying amounts. Therefore they will move at different rates in salt solution through which a current is passed. In paper electrophoresis the mixture to be separated is poured on a strip of paper after it has been moistened by the salt solution. Each end of the strip is put into a container filled with the solution. An electrode is submerged in each container and direct current passed through the solution. The different components migrate at different rates according to their electric charges. After they have been separated they are identified by various means. Electrophoretic approaches to the study of insect taxonomy have been attempted by Clark and Ball (1957), Brezner and Enns (1958) and Stephen (1961).

The polycellular evolution referred to above leads to the inference that the metabolic products of the cells constituting the different tissues must vary. While the same kinds of tissues from different insect species may exhibit apparently the same functions, histochemical differences between them may be observed which could be of taxonomic interest. Further infra-specific categorization may also be possible on this account. Histochemical approaches involve distinctive microtechniques and specific staining reactions. The mode of fixation of material for these studies is extremely important as there should be no chemical alteration from what exists in life. In the words of Gomori (1952) "fixation giving good cytological detail and minimum of artifacts should be preferred whenever possible. Often, however, a compromise is necessary; one may have to sacrifice cytological excellence to the preservation of the chemical substance investigated. This applies especially to the preservation of enzymes". Paraffin infiltration into whole insects for serial section cutting poses another major-problem. Usually because of the chitinous and more or less sclerotized integument invested with hairs, scales, *etc.*, in insects, the proper penetration of wax involves a much longer time than for dissected out tissues, leading to their 'cooking' and subsequent chemical alterations. Suitable techniques have, therefore, yet to be evolved to deal with even the histo-chemistry of individual insects, least to be said then of any histo-chemical assay from the taxonomic view point.

Often excreted products of insects contribute towards taxonomic segregation. Here, the historical background of the first introduction of the cochineal insect into India, which led to the spectacular control of the prickly-pear in the south, is particularly noteworthy. The early British settlers in this country were deeply fond of a deep crimson or red colour obtained from the mealy bug, *Dactylopius cacti*, from Mexico. Captain Nielson of the British Navy brought a live consignment of what he considered as these bugs to Calcutta in 1795 and handed them over to Dr. Roxburg of the Botanical Garden for colonising them on cacti in India and securing the dye locally. Unfortunately the dye obtained from these insects proved to be of an inferior quality because the species was quite different. It was *Dactylopius ceylonicus* and not *D. cacti* as supposed to be. Had Nielson known about this he may *not have taken the trouble to introduce it into India. It is* long after its cultivation was abandoned that its potential ability to control the spread of the dreaded prickly-pear could be realized. Thus the introduction of this insect ultimately proved a blessing in disguise. From this it may be observed that the taxonomic basis of many an excreted product of insects may be worthy of investigations. Lac, cantharidine, silk *etc.*, are of much socio-economic value and much could be done towards the refinement of such products through chemotaxonomic investigations.

Quite a different physiological approach in taxonomic segregation was attempted by Kulkarny (1964) under the author's guidance sometime back. This dealt with the *Aphis* *nerii* complex of aphid populations encountered with on two asclipidaceous plants belonging to the genera *Calotropis* and *Pergularia*. To the naked eye these differ in colouration: the *Calotropis* ones are deep yellow-ochre, whereas the *Pergularia* ones are yellowish green. In general morphology they look alike. To check up if the differential colours observed could be a mere optical illusion, due to the shade difference of the leaves of the two plants on which they occur, spectro-photometric studies were undertaken. The absorption bands produced by the ground-up homogenized extracts for adult apterous forms of the two populations were found to lie within entirely different ranges showing their distinctiveness. This observation was further strengthened through paper chromatographic and biometric approaches.

Saxena *et. al.*, (1965) have indicated that while a single biochemical character has often been considered for their taxonomic value by most investigators, it is desirable that a number of such characters are taken into simultaneous considerations in drawing taxonomic conclusions. It is worthy to note in this connection that Brown (1967) found that "the distributional data for the metabolic amino acid 3-hydroxy-L-kyneurenine as a wing pigment in nymphalid butterflies would suggest taxonomic groupings essentially in contradiction with those resulting from more broadscale morphological, breeding and behavioural studies; this indicates that the storage of this pigment is taxonomically relatively unimportant unit character". The selection of the biochemical characters that could be relied upon in physiological taxonomy, then, is as important as the selection of morphological characters.

Attention may now be focussed towards the utilitarian aspects of a physiological approach in insect taxonomy. Herein certain important biological principles are specially noteworthy. The basic tenet to be realized is that most of the biological associations met within nature are correlated with the metabolic activities of the associated organisms. Such associations involve one organism being either ectotrophic or endotrophic on another organism called the host. In the case of endotrophic forms all the nutritional sources must be necessarily present within the host. The metabolism of the organism associated in this way with any host may lead to beneficial or detrimental effects on the latter and accordingly the associated organism is stamped as a symbiont or a parasite. Physiological taxonomy of the various organisms, therefore, may be found to be useful to exploit this nutritional organic association fully to the benefit of mankind in various ways, especially in the field of insect parasitology and biological control of pests. In the case of ectotrophic forms too a chemotaxonomic approach may yield useful results as already shown by Brooke and Proske (1946) in determining the predators of immature mosquitoes through serological precipitin tests.

The physiological approach in Insect taxonomy has its useful repercussion on the chemical control of insects too. It may be borne in mind that by using a chemical insecticide what really takes place is interference with the normal physiological activity of the insect species involved to which it succumbs. From this, a toxicological approach in insect taxonomy also may be readily conceived.

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6

PENETRATION OF CHEMICALS THROUGH INSECT EGG-SHELL—A PHYSIOLOGICAL APPROACH TO OVICIDES

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The main object of this subject is to explain clearly the structure, chemical composition and modifications of egg envelopes during development because of their different environmental conditions, and to indicate how toxic chemicals penetrate from the outside to the inner living contents. It is not intended to devise methods of control or to obtain ovicidal materials, but to make explicit the broad principles governing the mechanism of penetration through insect eggs, and the protection provided to eggs of parasites developing within host-eggs. Such studies may help in selecting the ovicides effective against the eggs of pests, but not of parasites.

Morphology and physiology of insect egg-shell

In the eggs of *Corcyra cephalonica* Staint., *Chilo zonellus* Swinhoe (Lepidoptera), *Pyrilla perpusilla* Walk. (Homoptera), and external parasitic eggs of *Microbracon gelechiae* (Ashm.) (Hymenoptera), there is a remarkable parallelism in their fundamental structures to the eggs of *Pieris brassicae* Linn. (Fig. 6-1). The shell bears respiratory pores, running through its substance. It consists of external proteinaceous cement containing loose admixture of unsaturated oily substance, proteinaceous chorion made of non-resistant exochorion and resistant endochorion, and a lipoid layer of unsaturated oily material internal to the chorion in freshly laid eggs, and a mixture of unsaturated and saturated fatty substances in older eggs. The chorion and the lipoid layer are fully completed in the ovary. The latter is formed by the ovum and the former by the follicular cells. In eggs of *P. brassicae* and some of the eggs of *M. gelechiae* the lipoid layer is completed sometime after oviposition. The micropyle is closed by lipoid from this layer immediately after fertilisation and before oviposition, but in the eggs of *P. brassicae,* this happens within four hours after oviposition. The cement is deposited after fertilisation when the eggs are ready for oviposition. The proteinaceous vitelline and fertilisation membranes are devoid of any oil, but the serosa and later the epembryonic membrane contain a small quantity of unsaturated lipoid in older eggs, and it is re-absorbed by the developing larva before hatching.

The external waxy covering which is present on the batches of eggs of *P. perpusilla* gives them an additional advantage in resisting desiccation and penetration with solutions of aqueous materials. The proteinaceous cement of the eggs of these insects resembles that of the mite, *Metatetranychus ulmi* Koch (Beament, 1951) in having only a physical mixture of unsaturated lipoid with its substance but differs from that of *P. brassicae* in not containing any water-soluble reducing material linked to a protein. The cement of *M. gelechiae* like that of *Dialaraxia oleracea* Linn. (Lepidoptera) contains more lipoid than that found in the cement of other insects. The cement is non-adhesive and devoid of air spaces in *M. gelechiae* and *C. cephalonica*, whereas in *C. zonellus, P. perpusilla* and *P. brassicae* it is adhesive and spongy. The proteinaceous chorion of the eggs of these insects consisting of non-resistant exochorion and resistant endochorion is slightly different from that of *D. oleracea* in not having a layer corresponding to the thin exochorion of lipoprotein which covers the eggs. The respiratory pores in chorion of *C. cephalonica, C. zonellus, P. brassicae* and *D. oleracea* are somewhat concentrated at the anterior end of the eggs, whereas the pores are scattered all over the surface of the eggs of *M. gelechiae* and *P. perpusilla.*

Like the shell of the eggs of *Rhodnius prolixus* Stal. (Beament, 1948), the tick *Ixodes ricini* L. and *Ornithodorus moubata* Murry (Lees and Beament, 1948) which is not waterproof, the chorion of all these insects is freely permeable to quite big molecules of hydrophilic and lipophilic stains and chemicals in solution.

The lipoid layer on the inner side of chorion of the eggs of these insects is formed of freely mobile fatty material; some of its unsaturated lipoid is converted into saturated lipoid by the developing embryo whereas a hard wax has been indicated in eggs of *M. ulmi* (Beament, 1951), *R. prolixus* (Beament, 1946 a & b) and *Melanoplus differentialis* Thom. (Slifer, 1946). Such a lipoid layer has also been noticed in the eggs of *Locusta migratoria* Walker by Matthee (1951), but it has escaped the notice of Roonwal (1954) and Sanders (1956) in eggs of *Schistocerca gregaria* Forsk. and *P. perpusilla* respectively. Among the insects having lipoid layer, the transition temperature of eggs of *M. gelechiae* lies between 50°C. to 60°C. whereas that of other insects like P. *brassicae* between 60°C. to 70°C. The lipoid layer forms the main waterproofing mechanism of these eggs.

The non-waterproof chorion of the endoparasitic eggs of *Trichogramma evanescens minutum* Riley and *Tetrastichus pyrillae* Craw. differs from that of all the other insects in being very thin, consisting only of non-resistant protein, and

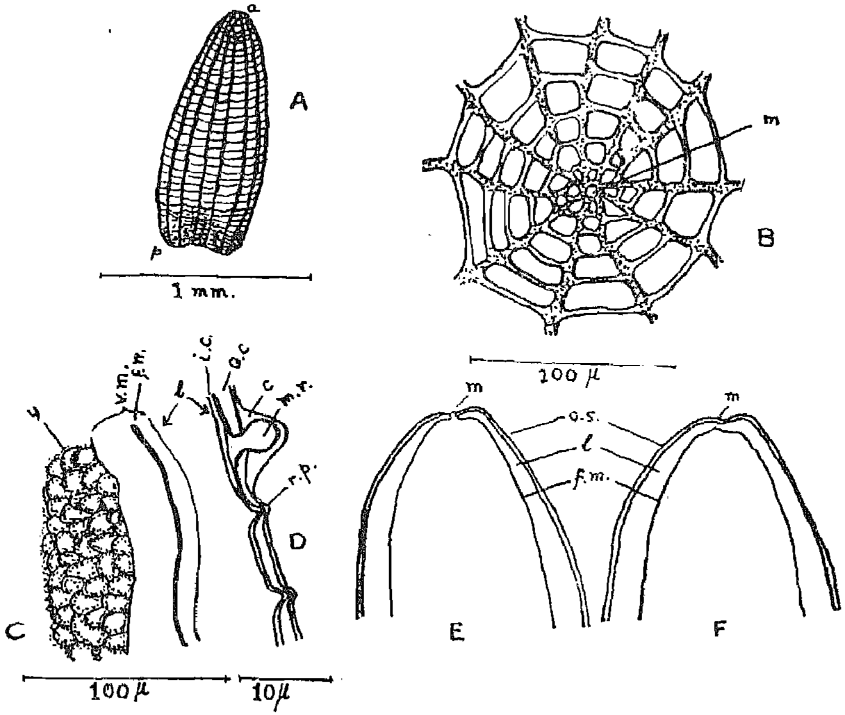

Fig. 6-1. Structure of the egg of *Pieris brassicae* Linn. A. whole egg showing anterior micropyler region (a) and flattened base (p). B. Anterior view of micropyler area and micropyler opening (m). C. Section through inner shell and yolk(y) at about two hours. The fertilisation membrane (f.m.) is developing in the vitelline membrane (v.m.). D. Section showing layers of the outer shell cement(c), inner chorion layer (i.c.), main rib (m.r.), outer chorion layer (o.c), the position of the lipoid layer(l) and of a respiratory pore(r.p), are shown. E. Diagrammatic representation of the fertilisation membrane (f.m.) applied to the micropyler opening (m) in a newly-laid egg. F. The same at six hours, showing the retraction of the yolk, allowing the lipoid(l) to flow in and isolate the outer shell (o.s.) and micropyle from the fertilisation membrane. (From Beament and Rattan Lal, 1957)

is devoid of respiratory pores. These eggs are also lacking in the internal lipoid layer.

The resistance of embryonic membranes of these insects to the action of corrosive chemicals is low in the early and late development, whereas it is more in mid-development. There is an incorporation of a little unsaturated lipoid in it at a latter stage, which serves to some extent as a secondary waterproofing mechanism. Beament (1949) and Matthee (1951) have also reported the formation of a secondary waterproofing mechanism in developing eggs of *R. prolixus* and *Locustana pardalina* respectively. In both these cases the secondary material is secreted by serosal cells, impregnating the secondary egg-membrane in the former egg, and giving a continuous film between the yellow and white cuticles in the latter egg. The epembryonic membrane of eggs of *T. evanescens minutum* and *T. pyrillae* is free from lipoid. Further in eggs of the parasites and the host insects it is ultimately reabsorbed by the developing larva sometimes before hatching (Rattan Lal, 1958).

Penetration of chemicals through egg-shell

The internal parasitic eggs are not resistant to the penetration of hydrophilic or lipophilic substances. The poison that gets into the host-eggs is enough to kill them. The eggs of other insects are remarkably resistant to water-soluble poisons except during the first four hours of development in case of *P. brassicae*. This resistance is attributed almost entirely to the lipoid layer and the early susceptibility of *P. brassicae* eggs to its absence over the micropyler region. These changes are not reflected in the effect of oil-soluble poisons or fumigants. The solid portions of the shell (cement and chorion) do not seem to be of great importance in restricting the entry of liquid poisons (hydrophilic or lipophilic); they often retain some poison to cause the death of those larvae while hatching; whereas all layers of the endoparasitic eggs are easily penetrated by these substances. It does not appear that the respiratory air spaces in the shell are preferential channels of access; rather, the poisons penetrate through the solid portions of the shell. This penetration, even of oil soluble materials is slow, for, they can be effectively washed out of the shell again some considerable time after dipping. On the other hand non-volatile oily materials can interfere with the respiration of the eggs by blocking the air spaces in the shell.

The obstruction to the penetration of water-soluble poisons is supplemented to some extent in mid-development when some fatty material appears in the epembryonic membrane and some of the unsaturated fat of the lipoid layer is converted into saturated state; at this time the eggs even start showing comparatively increased resistance to desiccation. The lipophilic substances penetrate quite easily through all layers of the eggs; but some resistance encountered by them and during mid-development may be due to the fully developed state of epembryonic membrane, which is resistant to the action of even some corrosive chemicals at this time.

The egg stage is commonly highly resistant to the usual contact poisons (Busvine, 1957). Pyrethrins and organophosphorus compounds may have some lethal action on eggs (as indicated by cessation of embryonic development). DDT and *gamma* BHC *etc.*, are virtually harmless to eggs although, being persistent, their residues often kill the young larvae on hatching; on the other hand lysol (phenol emulsion), dinitroanisole, mercuric chloride and hydrogen sulphide are highly toxic to eggs. Dinitrophenol compounds have been used extensively in the past to destroy eggs of insects and mites on fruit trees. The low susceptibility of insect eggs to contact poisons may be partly due to lack of penetration owing to the complex system of protective membranes surrounding the embryo, but many of the more effective contact poisons (like DDT, BHC, pyrethrins, organo-phosphorous compounds) act primarily on nervous function, and they would not be expected to attack embryos before the development of nervous system. Pyrethrins and TEPP have some lethal action on insect embryos which suggests that these poisons may have more generalised action at higher concentrations.

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7

INSECT INTEGUMENT

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The integument or body wall is that part of the ectoderm which externally surrounds the insect body and its appendages. It provides shape and rigidity to the animal body. It is a complex system containing several types of nerve receptors through which insect receives environmental stimuli and transmits them to the nervous system for eliciting proper responses. The outermost layer of body wall is made up of hard cuticular substances produced by the underlying epidermal cells. At several areas cuticle is further hardened or sclerotized to enable the body wall to function as exoskeleton to which are attached body muscles. The body wall is a counterpart of vertebrate internal skeleton with the difference that in insect the skeleton is external. It may be borne in mind that the entire body wall is not uniformly hardened but forms hard plates or rings called sclerites which are joined with one another by membranes. This arrangement enables the body to enjoy flexibility. The body wall, however, does not stretch except immediately after a moult. The membranous joints allow many types of movements of sclerites of abdomen (telescopic movement), articulation of legs, antennae and mouth parts, *etc.* The abdomen of an unfed female mosquito is very slender and elliptic but as the blood is sucked in, the abdomen enlarges due to the unfolding of pleated membranes at the pleural region as seen in cross section (Fig. 7-1). In grasshopper and locust the abdominal segments remain telescopically retracted (Fig. 7-2a) but these extend during oviposition when female lays eggs deep into the soil (Fig. 7-2b). How membrane of body wall helps in articulation at leg joints is illustrated in (Fig. 7-3a, b). When tibia is pushed up, the upper membrane is folded and lower is stretched. When tibia is pulled down the membranes behave in an opposite way.

The main function of insect body wall is to protect the animal from such environmental forces like evaporation, entry of disease-producing organisms and physical stress. It also, with the help of specialised sensory hairs and areas, appreciates the environmental changes to which the insect can advantageously orientate by moving or flying. Locomotion of this type is also connected to a great extent to the integument as most of the principal muscles are attached to internal surface of the body wall.

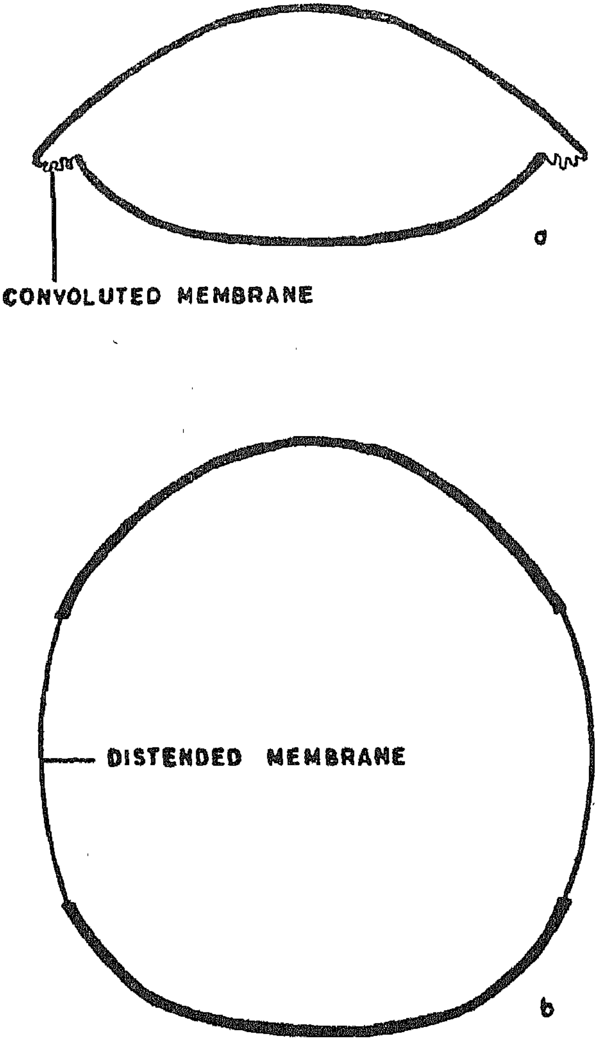

Fig. 7-1. Cross section of mosquito abdomen. (a) contracted (b) expanded.

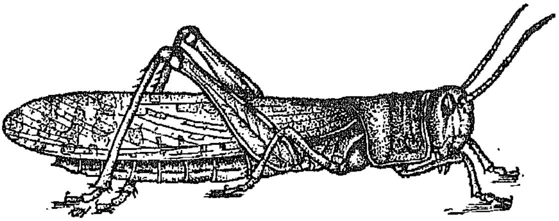

Fig. 7-2 a. Abdominal segments of locust in retracted position.

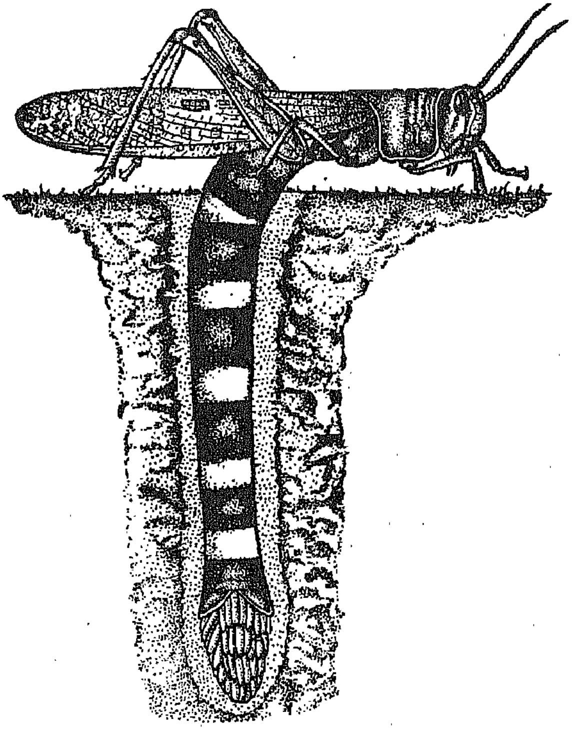

Fig. 7-2 b. Elongation of abdomen due to unfolding of intersegmental membranes joining the telescopic segments of locust during oviposition.

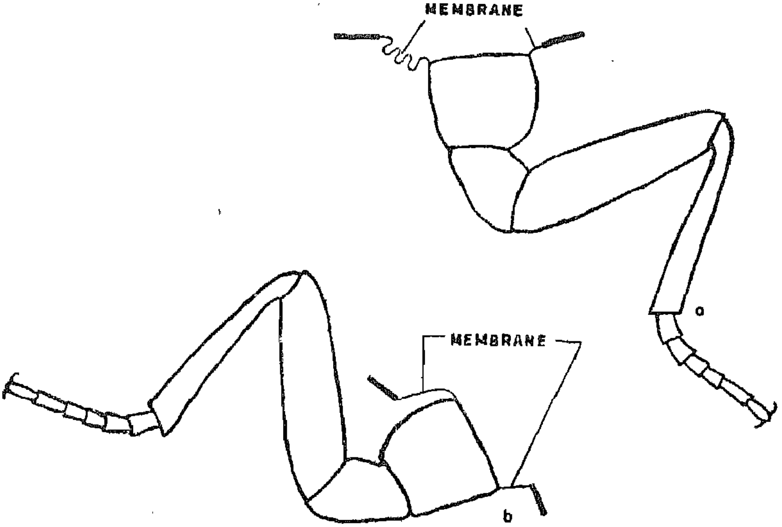

Fig. 7-3. Membranous folds in cuticle permitting articulation of appendages. (a) held straight (b) extended.

In certain regions of the body, the integument invaginates into haemocoele forming different types of internal outgrowths known by various terms depending upon the region where they occur. In the head of certain insects like cockroach, two or three pairs of inward growths known as apodemes, are found to which cephalic muscles are attached. These apodemes collectively form tentorium and lend support to brain and oesophagus. In thoracic region invaginations occur from tergal, pleural or sternal regions forming apodemes for the attachment of muscles. In abdomen such structures are not so much developed except in genital segment. This internal framework of head, thorax and abdomen has often been referred to as endoskeleton of insects by authorities like Imms and Snodgrass. Fox and Fox however hold a different view, which appears to be more reasonable. According to them no arthropod has an "endoskeleton". When muscles are attached to the outside of the skeleton the later is called endoskeleton as in vertebrates and when attachment is on internal the skeleton is called "exoskeleton". According to this definition even the so called "endoskeleton" of insects is in fact exoskeleton (tentorium and apodemes of thoracic and abdominal regions) because muscles are attached on the inner surface after piercing through the epidermis.

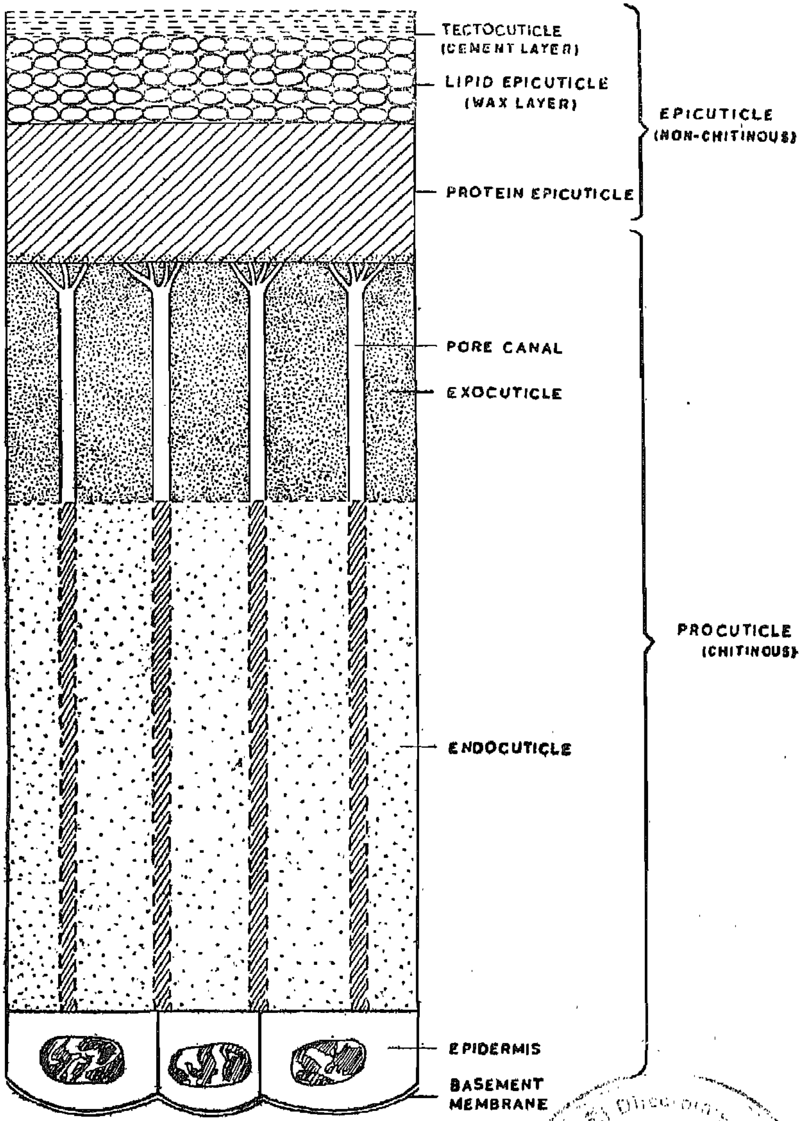

Fig. 7-4. Structure of the integument.

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STRUCTURE

The general structure of integument is shown in Fig. 7-4. It consists of three parts (i) cuticle, (ii) epidermis (hypodermis) and (iii) basement membrane. The cuticle is highly complicated and is variously modified and can be further subdivided into several layers of different chemical composition.

Cuticle

The epidermal cells secrete chemicals which polymerize to form cuticle. These substances are chitin, protein and lipid, which together form the cuticle. Chitin is one of its major constituents and is a nitrogenous polysaccharide. The cuticle has two subdivisions *viz.*, (a) epicuticle—non-chitinous and (b) procuticle—chitin-protein complex.

(a) Epicuticle: For a long time epicuticle was considered as a single layer. It has been shown that it too consists of 3 layers, depending upon the insect species. These layers are protein epicuticle consisting of protein and polyphenols, lipid epicuticle (also known as wax layer) and tectocuticle (also called cement layer). The epicuticle is very thin as compared to procuticle. Its thickness varies from a few hundredth of a micron (*Culex* larva) to four micron (*Sarcophaga* larva). The epicuticle is non-chitinous. During the formation of new cuticle oenocytes in the blood become highly active and supply material to epidermal cells for the secretion of protein epicuticle on the cell surface. Later polyphenolic substances are also incorporated in this layer. As development of cuticle proceeds the special glands in the epidermis produce a waxy material which in dissolved state is carried through the pore canals and deposited over protein epicuticle. This wax layer imparts water conserving property to the integument. Just before ecdysis or immediately after, cement layer is laid over as the outermost component of the epicuticle. The material of the cement layer is also secreted by special epidermal cells or glands.

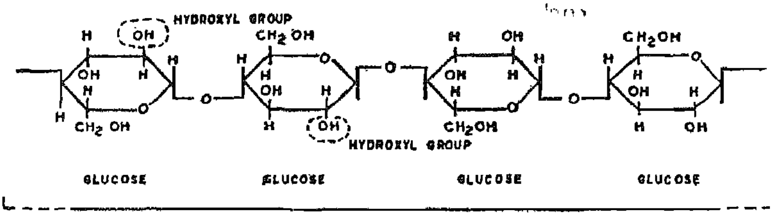
(b) Procuticle: The procuticle is comparatively much thicker layer and is composed of chitin protein and other substances. Chitin, however, is the chief constituent generally forming 20–50 per cent dry weight of the cuticle. It is highly resistant to alkalis and is a polysaccharide made up of chains of N-acetylglucosamine and glucosamine.

In some insects elastic rubber-like cuticle consisting of a special protein called "resilin" is present in articulatary sites of wings and mouth parts, helping in movement of these organs. The property of resilin is that it can be stretched in tension. As soon as the tension is withdrawn, the organ joined with resilin hinges comes back to its original position.

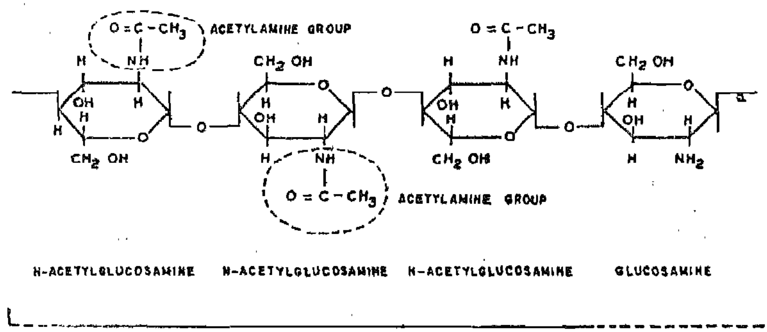
The procuticle can be distinguishable into two parts—outer exocuticle and inner endocuticle. Exocuticle is that part which is hard and dark in colour due to tanning and has several rigid sclerotized areas called sclerites. The endocuticle is the inner part and is not involved in tanning.

Important constituents of cuticle

1. **Chitin**: Chitin is a nitrogenous polysaccharide, insoluble in water and organic solvents and is resistant to even concentrated alkalis but not acids. When treated with strong acids, it hydrolyses to yield polysaccharides, glucosamine and acetic acid. The chitin is in the form of long chains consisting of several acetylglucosamines interposed by molecules of glucosamine. The adjacent chains are held by hydrogen bonds. The chitin molecule has a close resemblance to a cellulose molecule (consisting of large number of molecules of glucose), except that the chitin contains an acetylamine group instead of hydroxyl group at carbon 2 positions of the pyranose (glucose) ring as shown below:



CELLULOSE



CHITIN

Chitin, which is the main constituent of procuticle is found chemically linked with protein. The protein fraction is called arthropodin which is water soluble and lacks sulphur-containing amino acids. This protein undergoes tanning processes forming a hard, inflexible and dark sclerotin. Little is known about the precursors of chitin and the enzymes involved in its synthesis. It is speculated that chitin is derived from glycogen after it is degraded to its sugar components.

Presence of chitin can be demonstrated by chitosan test. The integument is treated with hot (160°C) concentrated alkali to produce chitosan which is soluble in dilute acetic acid and gives brown colour with iodine. This colour turns to violet with the addition of dilute sulphuric acid.

Besides insects, chitin is also widely distributed in the phylum Arthropoda where it is an important constituent of exoskeletal cuticle. It is also found in the egg shells of nematodes, in the bristles, jaws and gut lining of earthworms and other annelids. Cell walls of some fungi also contain chitin in the form of fungine.

2. **Lipids**: The integument has a covering of lipoidal layer consisting of wax. The thickness of wax layer varies 0.01–3 micron in different insects. It is secreted by special epidermal glands and the wax is perhaps transported in soluble or emulsified form to the site through pore canals. The grease layer on cockroach body is in fact the cuticular lipid. The wax layer helps preventing loss of water through the general body surface of insects. Water in the vapour form is lost as a result of passive diffusion, through areas on the cuticle from which lipid is removed artificially by organic solvents or abrasive dusts. In mosquito larva only the cuticle on anal papillae is permeable to water and salt.

Under high humid conditions, several species of insects and notably ticks can absorb water into the body by active absorption. In *Tenebrio* and *Leptinotarsa* integument has been shown to be permeable to water to some extent. Similar phenomenon perhaps occurs in all arthropods. It should be borne in mind that wax layer is a barrier through which water losses are minimum but this very barrier permits water intake from moist atmosphere by active transport of vapours, as absorption does not occur in dead insects. Water losses have been reported from dead and live insects suggesting a passive transport phenomenon.

3. **Polyphenols:** They play an important role in the formation of cuticle and imparting hardness to it . Tyrosine, an amino acid from the blood changes through a series of enzyme-controlled chemical reactions into phenols *e.g.*, dihydroxyphenol which is oxidized to a quinone. The quinone combines with cuticle protein to effect tanning and hardening.

Moulting and formation of new cuticle

The cuticle is hard and forms unstretchable exoskeleton in insect and it must be shed from time to time to permit the insects to increase in size during growth period. But before the old cuticle is shed, the new one has to be formed underneath it. The process of shedding the cuticle is called ecdysis. Moulting includes the series of changes, which developmental stages undergo for the formation of new cuticle and structures associated with it. The process of moulting ends by the accomplishment of ecdysis when the old cuticle splits to liberate the new instar of the insect. Moulting affects several organs of ectodermal origin, which besides integument include internal lining of fore and hind gut, taenidia of tracheae but not tracheoles of respiratory system *etc.* The pterygote insects cease moulting after attaining adulthood but mayflies undergo ecdysis soon after becoming adults.

Triggering of moulting by hormones

Triggering of moulting is under hormonal control. Essential features of this can be seen in the diagram. It has four main components as follows (Fig. 7-5):

(a) Neurosecretory cells in the brain under a suitable stimulus *e.g.*, blood meal in *Rhodnius* or temperature and light secrete material which is passed along the nerve axons to corpora cardiaca.

(b) Corpora cardiaca being a neurohaemal organ is closely associated with dorsal aorta. Through this organ the brain hormone is passed in circulating haemolymph and exerts its influence on prothoracic glands and corpora allata.

(c) Prothoracic gland when stimulated by brain hormone, produces a moulting hormone, ecdysone. Under the influence of ecdysone the cycle of growth and differentiation starts. The cuticle is the most susceptible tissue in this cycle.

(d) Corpora allata is also under the control of brain hormone, and it produces juvenile hormone. Juvenile hormone is not only essential in maintenance of prothoracic glands but also dictates the type of cuticle that is to be laid. When juvenile hormone is present in large quantity at the time of moulting, larval cuticle is formed while in its absence adult cuticle is formed.

Moulting fluid

The epidermis now separates from the cuticle giving rise to a narrow space in between the epidermis and cuticle. This process of separation of epidermis is called apolysis. This space is filled with moulting fluid secreted by epidermis. The fluid contains certain enzymes, chiefly a proteinase and a chitinase which digest the entire endocuticle but does not attack the epidermis. This is due to the two important steps which precede the digestion by moulting fluid. First, the epidermis, soon after separating from the cuticle starts secreting on its surface, the layer of protein epicuticle (which is

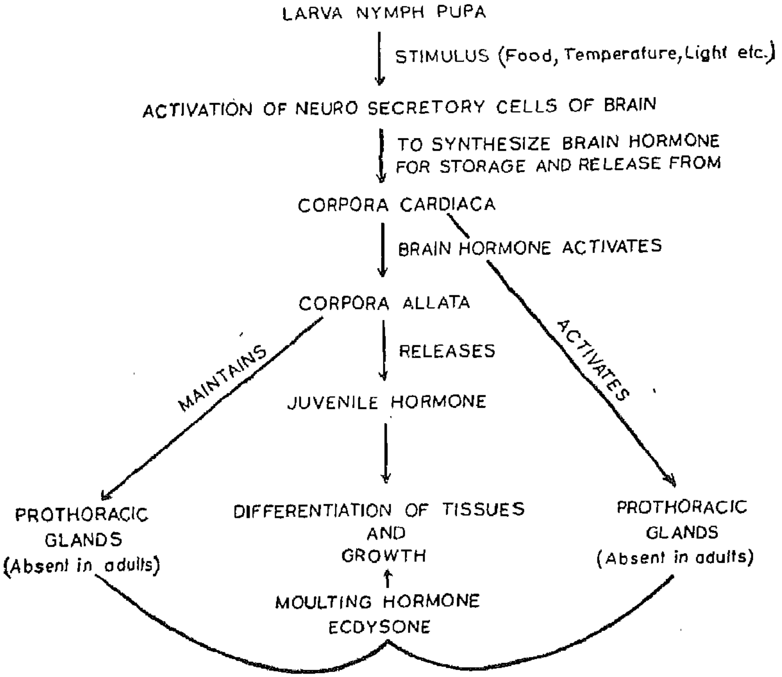

LARVA NYMPH PUPA
STIMULUS (Food, Temperature, Light etc.)
ACTIVATION OF NEURO SECRETORY CELLS OF BRAIN
TO SYNTHESIZE BRAIN HORMONE FOR STORAGE AND RELEASE FROM
CORPORA CARDIACA
BRAIN HORMONE ACTIVATES
CORPORA ALLATA
MAINTAINS
RELEASES
ACTIVATES
JUVENILE HORMONE
PROTHORACIC GLANDS (Absent in adults)
DIFFERENTIATION OF TISSUES AND GROWTH
PROTHORACIC GLANDS (Absent in adults)
MOULTING HORMONE ECDYSONE

Fig. 7-5. Triggering of moulting by hormones.

resistant to moulting fluid); secondly, the enzymes in the moulting fluid are activated only after the completion of the epicuticular layer.

The moulting fluid due to presence of some unknown phenomenon, has no effect on exocuticle, nerve and muscle connections to the old cuticle and a very thin portion of endocuticle. The latter forms the ecdysial membrane. The nerve and muscle connections are broken only at the time of ecdysis, till then the insect is able to move and to receive stimulus through the old muscle and nerve supply. The moulting fluid in the end is absorbed with about 90 per cent of the cuticular material. This is an excellent adaptation of converting and neutralising the nitrogenous and other chemicals of the old cuticle.

In insects which moult several times *e.g.*, some holometabolous larvae, the cuticle contains little exocuticle with the result that most of the cuticle is digested and absorbed. Some insects like Collembola eat their exuviae which is another method of conservation of cuticular material.

Ecdysis

After digestion of old cuticle and reabsorption of moulting fluid, the wax layer is laid on the surface of the cuticle. The insect shows active muscular activity to apply pressure on the old exocuticle which splits at ecdysial line of weakness. In many insects endocuticle at certain points extends up to epicuticle and these areas form ecdysial lines of weakness where the old skin splits. The muscular movements are supplemented by swallowing of air or water. In some dipteran pupa special organ called *ptilinum* is present on the head. This eversible sac is pushed out with a force to break the hard puparium when blood from abdomen and thorax is pumped into the head. When fly emerges the muscles of ptilinum bring the latter to normal position inside the head. Soon the head cuticles hardens, muscle attached to ptilinum degenerates and the sac is no longer eversible.

Once the cuticle splits, the new insect wriggles out. Head and thorax come out first followed by abdomen, legs and wings. Several invaginated cuticular parts are also shed for example, intimal lining of gut, taenidia of main tracheae, phragmata and tentorium *etc.* The wings are still not expanded. Insect swallows air and forces blood into wing veins for expanding them fully. The volume of insect also increases as the cuticle is not yet hard and is expandible. At this stage cement layer is laid over the wax layer. In due course of time cuticular parts are characteristically tanned and become hard.

Formation and deposition of cuticle layers

The first layer secreted over the surface of separated epidermis in the formation of the new cuticle, is protein epicuticle or cuticulin. Beneath it, is then deposited procuticle which after the tanning process, gets differentiated into exo- and endo- cuticle. In the next step, a waxy material is produced by the epidermal cells and is transported by pore canals through procuticle and deposited over the protein epicuticle in the form of lipid-epicuticle. Lastly, after ecdysis is complete, the cement layer is secreted as the outermost layer of epicuticle.

Thus, it can be seen that in the formation of epicuticular layers substance of innermost layer is secreted first and the materials of the outer layers are carried through pore canals and superimposed as successive layers described above.

Hardening of cuticle

When insect undergoes ecdysis the cuticle is soft and can expand to permit increase in the size of the animal. Soon procuticle consisting chiefly of protein and chitin undergoes chemical changes. The cuticular protein, arthropodin is water soluble. It becomes water insoluble as a result of tanning reactions. The tanning is brought about by the quinones derived from tyrosine, the concentration of which in the blood increases just before ecdysis and it falls to normal level when tanning reactions are complete. The tyrosine is converted to DOPA (dihydroxyphenylalanine) which changes to dopamine. These reactions occur in the special glands of epidermis. These substances which are at different stages of metabolism in different insects are carried through pore canal to the site just above the newly formed cuticle. Here they change to quinones. The quinone first tans the protein layer of epicuticle and then diffuses inwards tanning the protein in the outer part of the procuticle. This tanned part then becomes exocuticle. The tanned exocuticle becomes hard and brittle, it loses considerable amount of water and arthropodin changes into sclerotin. The tanning results in stabilization of cuticular protein whose molecules are packed together by cross-links formed by quinones.

Functions of cuticle

1. Cuticle acts as exoskeleton providing support to the body, its organs and imparts shape to the insect.
2. Muscles are attached to the cuticle to facilitate various types of body movements.
3. At several places cuticle is invaginated to provide internal support and muscle attachment.
4. Hard jointed legs with exoskeleton make efficient mechanism for movement with a minimum of muscle.
5. Wing movement is possible only because of the presence of hard cuticular flight sclerites in thoracic region.
6. The epicuticular wax layer provides a mechanism to conserve water in the terrestrial insects which otherwise would die of desiccation. Insects, due to their small size face this danger because the proportionate surface area increases as the body volume decreases.
7. Secretions over cuticular surface in some insects are bactericidal and protect insects from surface infection.
8. The cuticle is sclerotized to provide thick hard sternum which is subjected to abrasion by the sub-stratum. In beetles the thin tergum is protected with hard elytra.
9. The intima of fore and hind-guts protects epithelium. The peritrophic membrane in the midgut in some insects is a cuticular membrane secreted to protect naked epithelium.
10. Various parts of cuticle are modified to form sense organs to receive much needed environmental stimuli for proper orientation of body.
11. Cuticle also contains chemical pigments of various types or physical structures to produce metallic colour or iri-

descent colour due to reflection of light by the surface of scales in butterflies or granular surface of elytra of some beetles.

Pore canals

In the procuticle there are large number of ducts running from epidermis up to inner epicuticular layer. These are called pore canals. Electron miscroscopic studies have revealed that these are helical rather than straight tubes (Fig. 7-6.). The number of pore canals per unit area varies in different insects. In *Sarcophaga* 50–70 canals arise from an epidermal cell giving a density of 15,000 per sq. mm. In cockroach the number is 1,200,000 per sq. mm. The function, structure and fate of pore canal during development is imperfectly understood. In some cases protoplasmic strands run from the epidermal cell, suggesting that the canal acts as a means to transport material during the formation stage of cuticle. They probably carry moulting fluid, protein, polyphenols and perhaps wax.

Pore canals are absent in inner endocuticle but can be seen only in outer endocuticle in newly moulted insect. Often lumens of pore canals are blocked by cuticular material. In

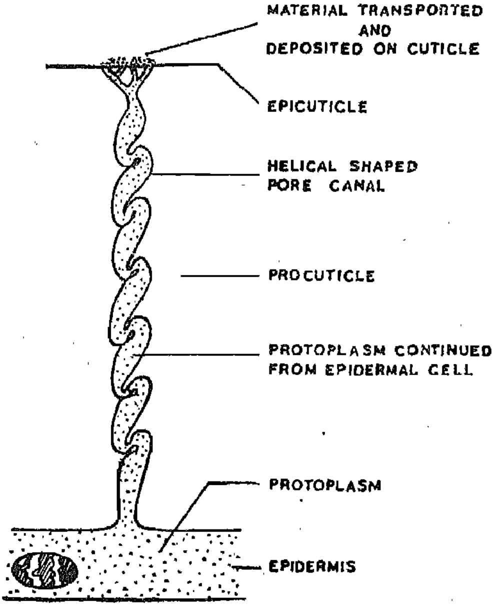

MATERIAL TRANSPORTED AND DEPOSITED ON CUTICLE
EPICUTICLE
HELICAL SHAPED PORE CANAL
PROCUTICLE
PROTOPLASM CONTINUED FROM EPIDERMAL CELL
PROTOPLASM
EPIDERMIS

Fig. 7-6. Structure of a pore canal.

such cases materials are deposited outside not through pore canals but by a diffusion process through the endocuticle.

Epidermis (Hypodermis)

It is one cell thick and underlies the cuticular part. Some of the cells are variously modified to form sense organs (chemo-tacto-, hygro- and photo- receptors). The peristigmatic glands surrounding the spiracles are also specialized epidermal cells. When muscles are attached to exoskeleton the former penetrate the epidermal cells and are connected with the procuticle through specialized fibres called tonofibrillae. It is often suggested that the oenocytes of blood arise from epidermal cells and are involved in intermediary metabolism of substances required during formation of new cuticle. Some of the cells are enlarged to form glands which open outside through a duct. These glands are of various types producing variety of chemical substances like cuticular materials, moulting fluid and poisons for defensive purposes. The poison glands normally open into a seta-like structure, the tip of which when breaks releases the poison to ward off enemies.

Basement membrane

The epidermis rests upon on undifferentiated basement membrane which is about 0.5 micron thick. It is often continuous with the basement membrane of sarcolemma. Some workers have stated that the basement membrane is a type of connective tissue but so far histological evidences have failed to support the cellular nature of this membrane.

Cuticular appendages and processes

General surface of the integument shows various types of specialized outgrowths like setae, spines, spurs *etc.* Setae or *macrotrichia* are hair like projections which arise from special epidermal cell called *trichogen* which penetrates through another cell called *tormogen* which at the outside forms a membrane. The seta is attached to this membrane and articulates at this site. At each moult the cuticular spine of macrotrichia is renewed. In several cases the seta like structure innervates the sense organ formed by the epidermal cells which receive sensory nerve.

Besides this, spines, tubercles, spurs and fixed hairs or microtrichia are present as fixed cuticular processes which are rigidly connected with the cuticle having no membranous articulation. These processses are multicellular in origin unlike setae which are unicellular.

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8

INSECT WINGS AND FLIGHT

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Insects are the only animals possessing true wings which constitute the characteristic features of the class Insecta. The power of flight has given them an obvious advantage and has helped them to dominate the animal kingdom (See chapter 4). In animals like birds and bats, power of flight has been attained at the cost of loss of fore limbs used for locomotion on the ground. In insects such a situation does not exist as wings have developed independently as lateral expansions of the body wall of thoracic segments. Development of wings as powerful locomotory organs has brought about various complex modifications in sclerites of meso- and metathorax and their internal musculature for providing necessary mechanism for wing movements.

Wings are usually present in adult insects but a number of them are also known to be wingless (apterous).The apterous condition is a primitive characteristic and is a constant feature of the subclass Apterygota. In Pterygota this is a secondarily acquired character as in members of all the parasitic orders like Mallophaga, Siphunculata, Siphonaptera; sterile castes of termites and ants; female coccids; female stylops and embiids. Among other Pterygota this condition is of a casual occurrence and is often confined to a single sex or species. However, transitory forms are also found in some groups.

Generally, insects possess two pairs of wings borne by the meso- and metathorax. The first pair of wings in Strepsiptera, and hind pair in Diptera and male Coccoidea are reduced to short club-like processes which act as balancers during flight. In Diptera these are known as halteres (Fig. 8-1e). The fore pair' of wings in Coleoptera are called elytra which are sclerotized and horny and form a protective covering over the hind pair (Fig. 8-1d). In Hemiptera these are partly thickened and partly membranous and are termed hemelytra (Fig. 8-1b). Orders like Orthoptera, Dictyoptera and Phasmida are characterized by the presence of leathery fore wings known as tegmina (Fig. 8-1a). The names of most of the Pterygote orders have also been derived on the basis of nature of wings, for instance, Orthoptera (straight + wings), Isoptera (equal + wings), Lepidoptera (scale + wings), Coleoptera (sheath + wings), Hymenoptera (membrance + wings) and Diptera (twice + wings), *etc.*

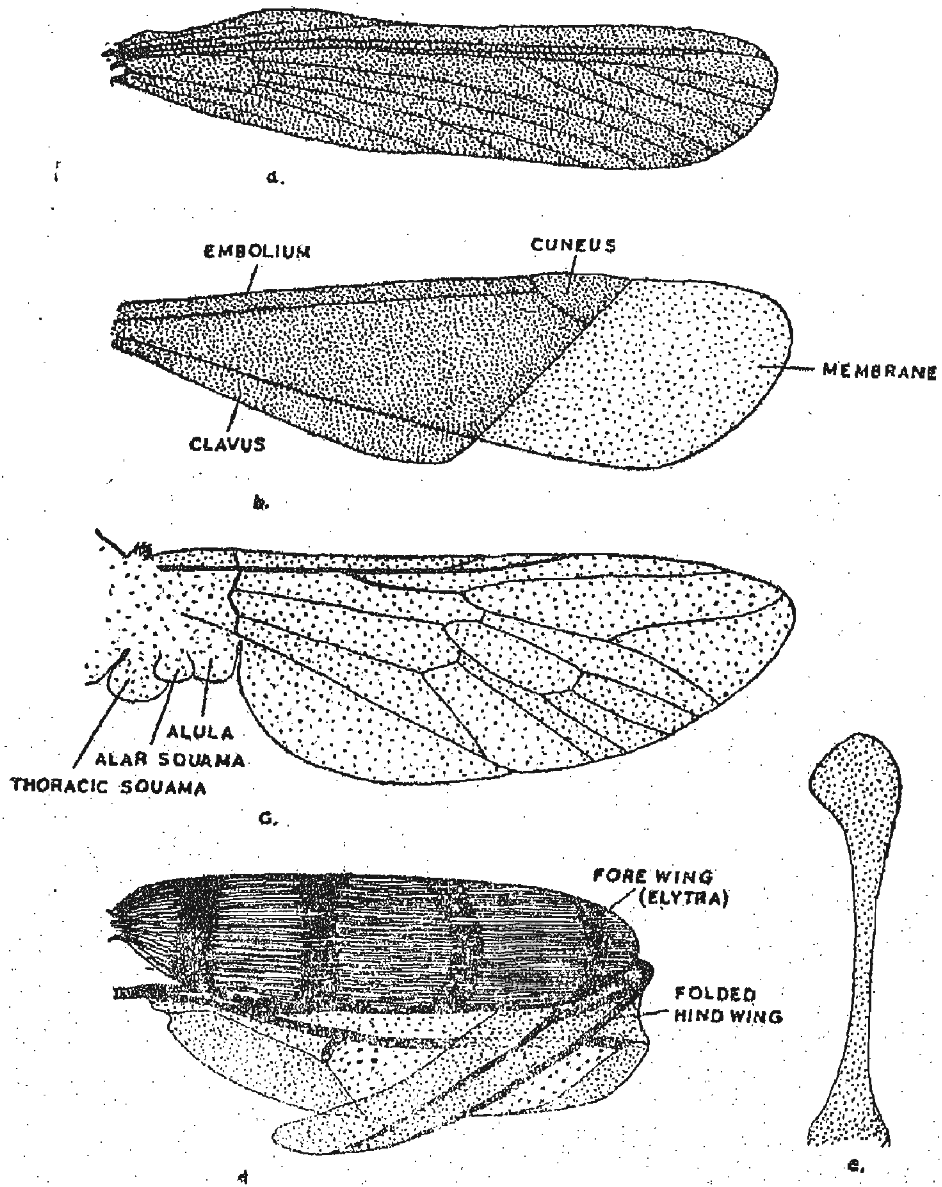


Fig. 8-1. Different types of wings (a) fore wing of Orthoptera (b) forewing of Hemiptera (c) fore wing of Diptera (d) fore and hind wings of Coleoptera (e) hind wing of Diptera.

The way in which wings and flight were evolved in insects, has not so far been understood clearly as the available fossil evidence fails to throw any light on the possible precursors of modern wings. Two principal theories have been put forward about the origin of wings in insects.

(*i*) *The tracheal gill theory*. It emphasizes that the wings were derived from thoracic tracheal gills which later became adapted for purpose of flight. This view is now no longer acceptable since the development of tracheal gills is very inconsistent. They may develop from tergum, sternum or pleuron, at apex of abdomen, on head or even in between the wings. Moreover, one has to presume that the ancestors of insects were positively aquatic—a fact which is not true.

(*ii*) *The paranotal theory*. It suggests that wings probably arose as lateral expansions of thoracic body wall. In the beginning these were of little or no use for aerial mode of life; later they probably evolved further as gliding apparatus with the help of which ancient insects could glide through the air from a high to low elevation. It is likely that during the course of evolution these expansions became flexible, so that the internal thoracic muscles could change the plane of flaps to enhance and to direct the glide.

In order to have a clear understanding of the mechanism of flight, it is first necessary to gain some basic knowledge of the structure of insect wing, its basal articulation with the body and the arrangement of various flight muscles in the thorax. Flight in insects is actually the net results of a combind action of these elements together with certain other activities like the respiration, circulation of blood and sensory phenomena.

THE STRUCTURE OF WINGS

Insect wings are outgrowths of the integument, arising as flattened sac-like evaginations at the dorso-lateral margins of meso- and metathorax, collectively known as pterothorax. The wings being a derivative of the ectoderm, consist of cuticle, epidermis and the basement membrane. During the development the wings appear as small sac-like outgrowths of the integument (Fig. 8-2a). Its hollow space is filled with body fluid, nerves and tracheae. At certain areas cuticle thickens to form future veins (Fig. 8-2b). Later on the epidermal cells elongate pushing the basement membrane in such a way that the membranes from two apposing layers unite to form what is some time referred to middle membrane of the wing (Fig. 8-2c). At several places however where the cuticular thickenings arise the basement membranes do not meet, forming empty spaces which in some forms may determine the course of future veins. It may however be mentioned here that according to some authorities characteristic tracheation in immature forms arises from the tracheae located at the base of wing. These tracheae in fact enter the channels described above. The wing veins are therefore in direct communication with the tracheal branches for the air supply, with the nervous system for the functional sensory organs situated on the wings and with the haemocoele for efficient blood supply. Another view on the origin of the venation is that it arises as thickenings of the wing channels independently of tracheae. This view however has not received much acceptance.

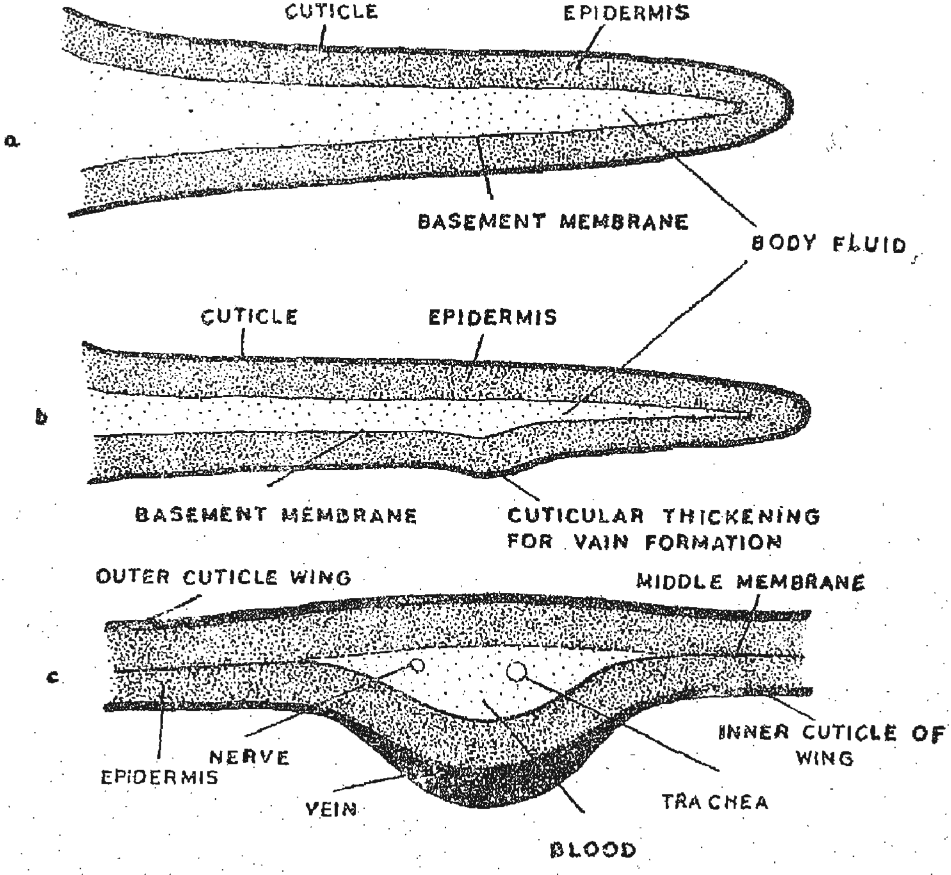


Fig. 8-2. Various stages' of a developing wing. (a) Sac-like evagination from the thoracic wall (b) flattening of wing and cuticular thickening for vein formation (c) apposing basement membranes unite to form middle membrane, thichened region transformed into a vein.

A generalized insect wing is more or less triangular membranous flap, strengthened by a number of veins arising from its narrow base and running towards the outer margin (Fig. 8-3). The wings may be further reinforced by the presence of several cross-veins. The number of veins and cross-veins is highly variable among various orders of insects. The region of wing containing bulk of the veins is called the remigium, behind which lies another region called vannal or anal separated by vannal or anal fold. Proximal to the latter in some insects, is another lobe called the jugum separated from vannus by a jugal fold. In Diptera there are three other lobes known as thoracic squama, alar squama and alula (Fig. 8-1c).

The anterior margin of the wing is called the coastal margin, outer as apical margin and the posterior as the anal margin (Fig. 8-3). Similarly the angle at the base of the wing is called the humeral angle, the one between coastal and apical margin as the apical angle and between apical and anal margin lies the anal angle. The anterior longitudinal veins are generally heavier and more closely arranged than the posterior veins. The wing rotation is facilitated as a result of this difference in the consistency of wing-regions.

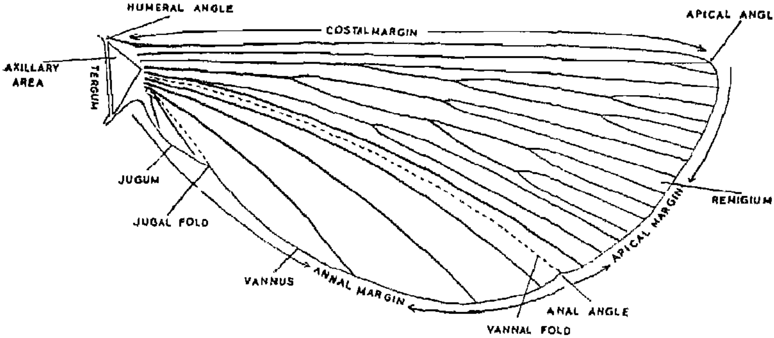


Fig. 8-3. Insect wing, its venation, margins, angles and areas.

THE ARTICULATION OF WINGS

The articular membrane with which the wing is moveably attached with the tergum is provided with sclerites collectively known as pteralia (Fig. 8-4). This region forms the axillary area of the wing (Fig. 8-3). These not only help in wing movement but also provide the flexor mechanism in all insects except Ephemeroptera and Odonata which do not flex their wings over the abdomen at rest. The basal sclerites or pteralia including the following:

The humeral plate: It is a small sclerite anteriorly situated at the base of the wing and moveably articulated with the base of the costa.

The first axillary: This sclerite forms the anterior hinge plate of the wing base. Its anterior basal margin is supported

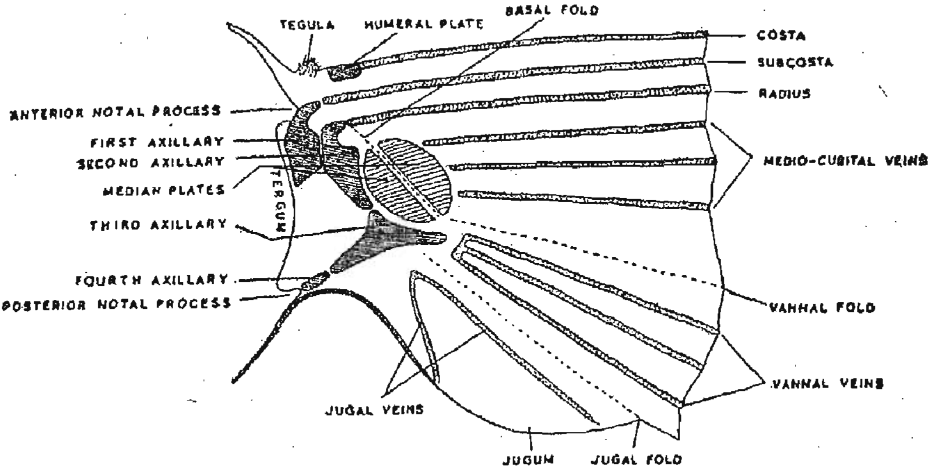


Fig.. 8-4. Basal sclerites of wings.

on the anterior process of the tergum which is called the anterior notal process whereas the posterior basal margin articulates with the tergal margin. The outer or distal anterior part is usually produced into a slender arm, associated with the second vein of the wing, the subcosta and the posterior distal end articulates with the second axillary.

The second axillary: This is the pivotal sclerite of the wing base as its ventral surface rests upon the fulcral wing process. Its anterior basal margin articulates with the first axillary, whereas the knob-like outer end manipulates the radial vein. The posterior end articulates with the mesal arm of the third axillary.

The third axillary: Its shape is highly irregular. Generally it is provided with three arms, *viz.*, the mesal end articulating with the second axillary, posterior end with the posterior notal process and the outer or distal end manipulates a group of veins present in the basal region of the wing collectively known as vannal veins. This sclerite has a special significance because the flexor muscles of the wing are inserted on its inner margin.

The fourth axillary: This sclerite whenever present intervenes between the posterior end of third axillary and the posterior notal process. It is regarded as a detached portion of the third axillary.

The median plates: Usually there are two semi-circular plates lying in the median area of the wing base, distal to second and third axillary. The proximal plate is usually attached to the distal arm of the third axillary whereas the distal plate represents a general sclerotization at the base of medio-cubital veins. These two plates are separated by an oblique line which forms a prominent convex basal fold.

THE WING MUSCLES

In majority of insects the wing movement is brought about by a set of five pairs of muscles present in each of the wing bearing segments.

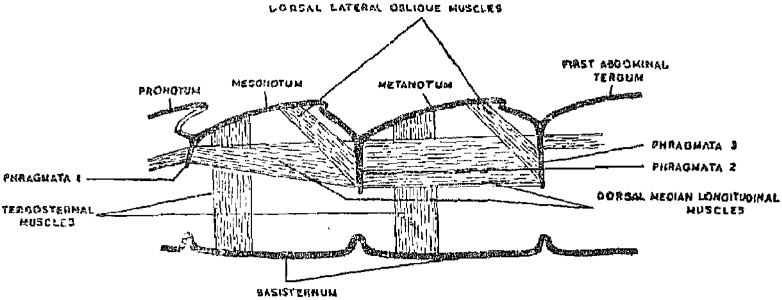


Fig. 8-5. Dorsal and tergosternal muscles of pterothorax.

The dorsal muscles (Fig. 8-5) : The dorsal muscles comprise of the following sets of muscles.

(a) *Median longitudinal muscles.* In meso- and metathorax the ordinary longitudinal muscles of the dorsal region become greatly enlarged to contribute in the motor mechanism. These are attached to special lobes called the phragmata which are the invaginations of exoskeleton along the intersegmental lines between pro- and meso-, meso- and meta, and meta- and 1st abdominal terga. Contraction of these muscles causes an upward arching of the wing-bearing terga in between its two ends, resulting in a downward deflection of the wings on the pleural fulcra.

(b) *Lateral oblique muscles.* These muscles arise on the posterior part of the scutum and are inserted on the succeeding phragmata. In function they supplement the tergosternal muscles in their downward pull.

The tergosternal muscles (Fig. 8-5) : The tergosternal muscles arise on the anterior lateral areas of the tergum and are inserted ventrally on the basisternum before the coxae. The contraction of these muscles results in the depression of the wing bearing tergum in between its two ends, thereby elevating the wings on the pleural fulcra. In some insects the function of the tergosternal muscles in elevating the wings is supplemented to some extent by other muscles.

The axillary muscles (Fig. 8-6) : Two sets of these muscles may be recognised which arise on the pleuron and are inserted on the first and third axillary sclerites. The one which is inserted on the first axillary is known to occur only in Diptera. A pull on these muscles turns the sclerite upward on its tergal articulation thereby helping in the flexion of the wing.

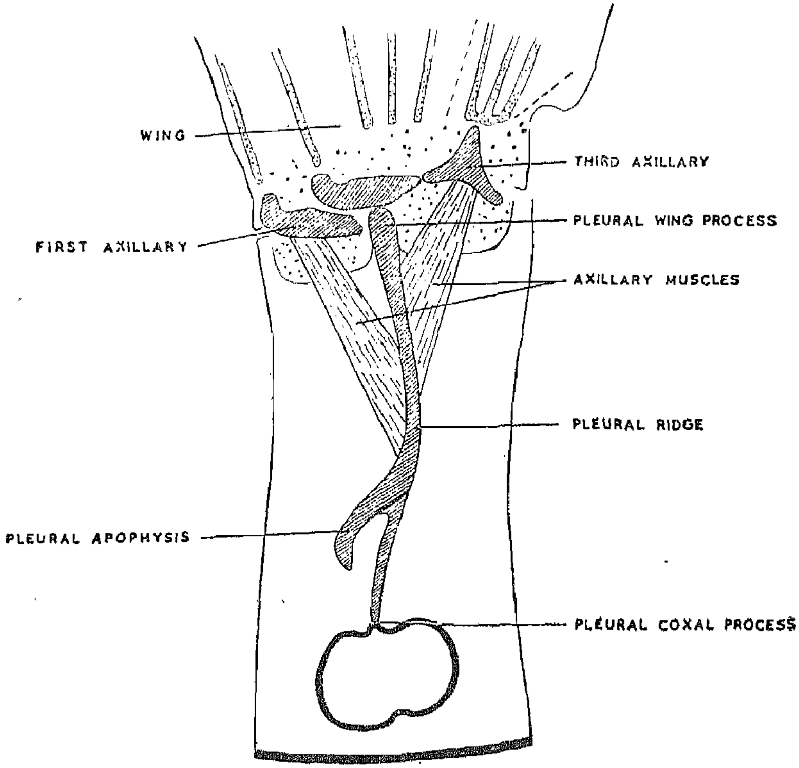


Fig. 8-6. Axillary muscles.

The muscles inserted on the third axillary are responsible for the complete flexion of wings over the abdomen at rest hence are present in all the wing flexing insects. A pull on these muscles revolves the third axillary dorsally and inward on its mesal and proximal articulation thereby turning the wing posteriorly on the axillary region along the basal fold present in between the two median plates. In Odonata which do not flex their wings, these muscles and the corresponding sclerites are absent. In Ephemeroptera these muscles though present are not functional.

The basalar muscles (Fig. 8-7): One to three pairs of these muscles are generally present which arise on the episternal wall of the pleuron of wing bearing segment and are inserted on a sclerite called the basalare embedded in the articular membrane at wing-base anterior to the pleural wing process. In some cases these may also arise on the outer rim of coxa anterior to the pleural coxal articulation or on the trochantin or on the sternum itself. The function of these muscles is to depress the costal margin of wing during flight and also to extend the flexed wing because the basalare sclerite is in close connection with the humeral angle of the wing. For this reason these muscles are also called the pronator-extensor muscles.

The subalar muscles (Fig. 8-7): These muscles are the counterparts of basalar muscles as they arise on the epimeral wall of the pleuron and are inserted on a sclerite called the subalare embedded in the articular membrane at wing base posterior to the pleural wing process. Like the basalar these muscles may also arise on the coxa at its meral lobe posterior to the pleural coxal articulation or on the sternum itself. Since the subalare sclerite has close connection with the second axillary, the subalar muscles serve to extend and to depress the wing and on this account are also called the depressor-extensor muscles of the wing.

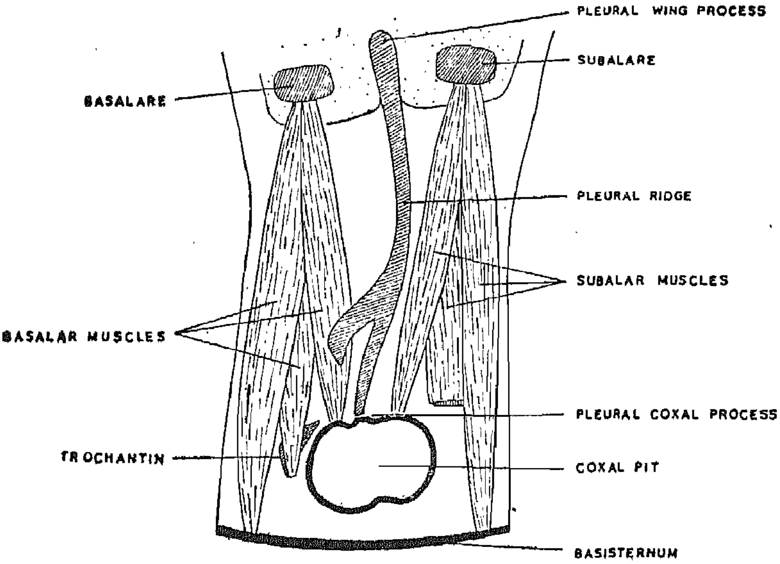


Fig. 8-7. Basalar and subalar muscles.

In insects which may be relatively weak fliers, the percentage of flight muscles in proportion to the body weight is low. For instance in *Oedipoda* (Orthoptera) they comprise only eight per cent of the total body weight. In strong fliers this proportion is far greater as for example in *Musca* (Diptera) it is 11 per cent, in *Apis* (Hymenoptera) 13 per cent, in *Macroglossa* (Lepidoptera) 14 per cent and in *Aeschna* (Odonata) as high as 24 per cent of the total body weight.

THE WING COUPLING IN INSECTS

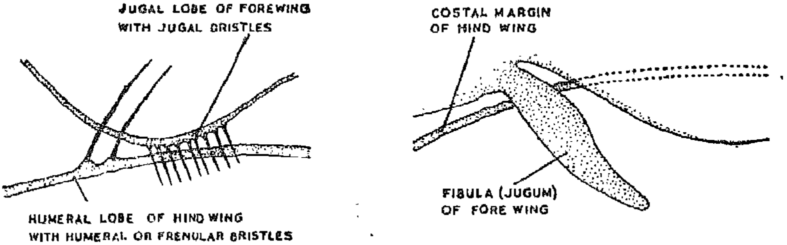
The development of the wing coupling in insects is a device through which they have evolved a better method of using their wings during flight. Among the more primitive insects the two pair of wings move independently of each other but in others, there appears to be a tendency for using them as a single functional unit. This is achieved either by reducing one pair of wings as in Diptera or by devicing a wing-coupling apparatus as described below:

A primitive type of wing coupling occurs in family Choristidae (Mecoptera) which perhaps has given rise to other types. Here the posterior basal region of fore wing and the anterior basal region of hind wing are developed into special lobes called the jugal and humeral lobes respectively. These are provided with comparatively longer bristles which according to their positions, are known as jugal and humeral bristles. The jugal bristles lie on top of the hind wing while humeral ones press against the underside of the fore wing, thus keeping the two wings intact during flight (Fig. 8-8a). In Trichoptera only the jugal lobe is present which lies on top of the hind wing during flight. This lobe is relatively longer in the family Hepialidae (Lepidoptera) and is called fibula which remains folded beneath the fore wing during rest (Fig. 8-8b).

Many Lepidoptera are provided with a frenate wing coupling which slightly differs in the two sexes. In female (Fig. 8-8c) the humeral lobe of hind wing is provided with a group of long bristles, the frenulum which interlocks with the retinaculum composed of several forwardly directed hairs on the underside of the cubital vein of fore wing. In males (Fig. 8-8d) however, there is a single stout frenular bristle interlocking with a single stout downwardly curved spine of retinaculum on the underside of the radial or subcostal vein.

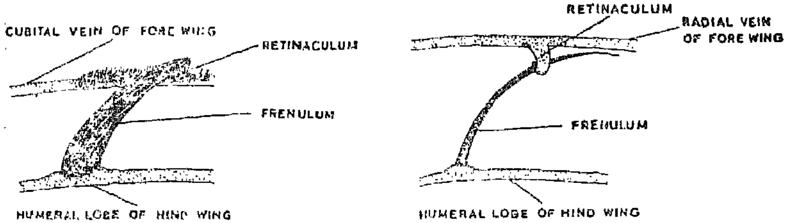
In the superfamilies Papilionoidea and Bombycoidea of Lepidoptera the wing coupling is effected by a basal overlapping of the broad fore and hind wings and is called the amplexiform wing coupling (Fig. 8-8e).

Hymenoptera are characterised by a hamulate wing-coupling (Fig. 8-8f) where a row of curved small hooks, the hamuli are present along the costal margin of hind wing which interlock with the thickened hind margin of the fore wing. In


JUGAL LOBE OF FOREWING WITH JUGAL BRISTLES
HUMERAL LOBE OF HIND WING WITH HUMERAL OR FRENULAR BRISTLES
COSTAL MARGIN OF HIND WING
FIBULA (JUGUM) OF FORE WING

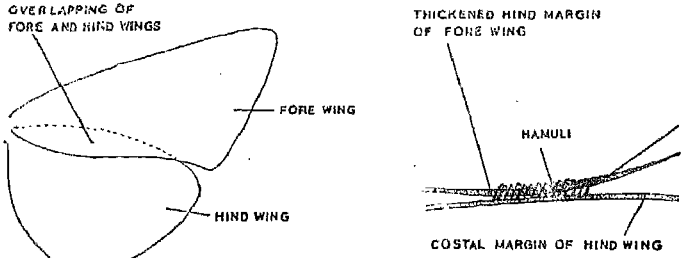
a. JUGO-FRENATE WING COUPLING (MECOPTERA)

b. JUGATE WING COUPLING (TRICHOPTERA AND PRIMITIVE LEPIDOPTERA)


CUBITAL VEIN OF FORE WING
RETINACULUM
FRENULUM
HUMERAL LOBE OF HIND WING
RETINACULUM
RADIAL VEIN OF FORE WING
FRENULUM
HUMERAL LOBE OF HIND WING

c. FRENATE WING COUPLING (FEMALE LEPIDOPTERA)

d. FRENATE WING COUPLING (MALE LEPIDOPTERA)


OVERLAPPING OF FORE AND HIND WINGS
FORE WING
HIND WING
THICKENED HIND MARGIN OF FORE WING
HAMULI
COSTAL MARGIN OF HIND WING

e. AMPLEXIFORM WING COUPLING (LEPIDOPTERA)

f. HAMULATE WING COUPLING (HYMENOPTERA)

Fig. 8-8. Wing coupling in various insects.

many Hemiptera the wing coupling is brought about by means of various small hooks or folds along the wing margins.

THE MECHANISM OF WING MOVEMENTS

The movement of wings for purpose of flight involve two distinct mechanisms, the indirect and direct. In majority of insects the flight is chiefly effected by indirect method when muscles deform the pterothorax which results in inducing the upstroke and the downstroke of the wings. However, this movement may also be supplemented by a direct method when muscles inserted at base of wing cause the forward and backward strokes and partial rotation of wing on its long axis. These direct muscles of flight are predominently operative in Blattoidea and remain important in all Orthoptera, Coleoptera and Odonata.

The up stroke of wings: When the tergosternal and oblique dorsal muscles contract, the tergum of the wing-bearing segment gets depressed with the result that the wings are elevated on the pleural fulcra thereby causing the upstroke of the wing (Fig. 8-9a). Here the wing acts as a lever of the first class with the wing process as fulcrum between the force and the load. Since the tergosternal muscles are mostly powerful the upstroke is very important in the mechanism of flight.

The down stroke of wings: *The depressed tergum of* the wing-bearing segment is arched upward by exerting a pull on the dorsal longitudinal muscles which results in the depression of wings (Fig. 8-9 b, c). The downstroke is made further powerful by supplementing a direct pull by the subalar muscles.

Elasticity of thorax helping the up and down strokes: The up and down strokes are greatly helped by the elastic properties of the thorax. When the dorsum is depressed by the pull of dorsosternal muscles and the wings are lifted, the lateral walls of the thorax get expanded. This expansion is resisted by the elasticity of the thorax which is due to the pleurosternal muscles present in between the pleural and sternal apophyses (internal processes arising from the pleural and sternal ridges) (Fig. 8-10) of all insects except the mesothorax of Coleoptera and metathorax of Hymenoptera. On account of this elastic stiffness the position of wing is stable only when they are fully up or fully down. Any position in between will be unstable and the wing will tend to return to any of the stable positions by an abrupt movement. This arrangement is called the 'click' mechanism and is greatly helpful in accelerating the wing motion with little input of energy, the rest being stored as elastic force to be utilized during the reverse process.

The anteroposterior and rotary movement of wings: The direct movement of wings is caused by various sets of muscles inserted on two sclerites present at the base of the wing before and behind the pleural wing process. The resulting movements include a forward movement, a backward movement and a partial rotation of each wing on its long axis.

Anterior and posterior deflections of the wings are effected during down stroke and up stroke respectively. When the wing

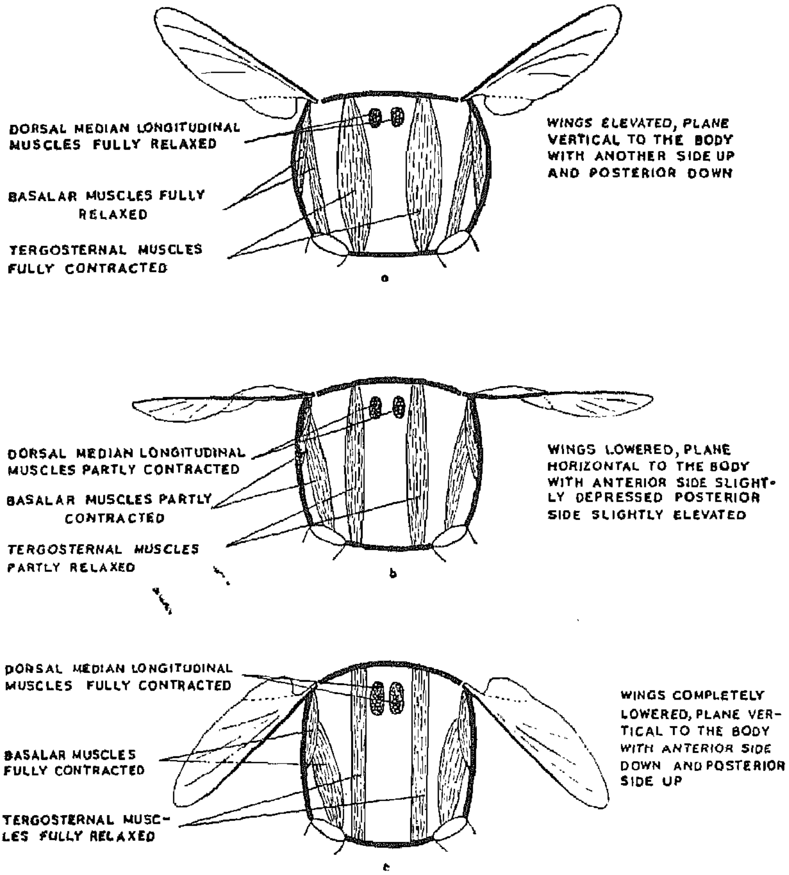

DORSAL MEDIAN LONGITUDINAL MUSCLES FULLY RELAXED
BASALAR MUSCLES FULLY RELAXED
TERGOSTERNAL MUSCLES FULLY CONTRACTED
WINGS ELEVATED, PLANE VERTICAL TO THE BODY WITH ANOTHER SIDE UP AND POSTERIOR DOWN
a
DORSAL MEDIAN LONGITUDINAL MUSCLES PARTLY CONTRACTED
BASALAR MUSCLES PARTLY CONTRACTED
TERGOSTERNAL MUSCLES PARTLY RELAXED
WINGS LOWERED, PLANE HORIZONTAL TO THE BODY WITH ANTERIOR SIDE SLIGHTLY DEPRESSED POSTERIOR SIDE SLIGHTLY ELEVATED
b
DORSAL MEDIAN LONGITUDINAL MUSCLES FULLY CONTRACTED
BASALAR MUSCLES FULLY CONTRACTED
TERGOSTERNAL MUSCLES FULLY RELAXED
WINGS COMPLETELY LOWERED, PLANE VERTICAL TO THE BODY WITH ANTERIOR SIDE DOWN AND POSTERIOR SIDE UP
c

Fig. 8-9. Mechanism of wing movements.

comes down during down stroke, the muscles of the basalare are pulled which turn this sclerite inward on the upper edge of the episternum with the result that its connection with the humeral angle causes deflection of the anterior part of the wing which slightly turns forward (Fig. 8-9c).

In the reverse movement when the wing comes up the air pressure on the expanded membranous posterior region of the wing causes a backward deflection which is further strengthened by a tension on the subalar muscles, exerting on the second axillary.

As we have already seen, the anterior parts of wings are heavier due to crowding of veins and the posterior larger

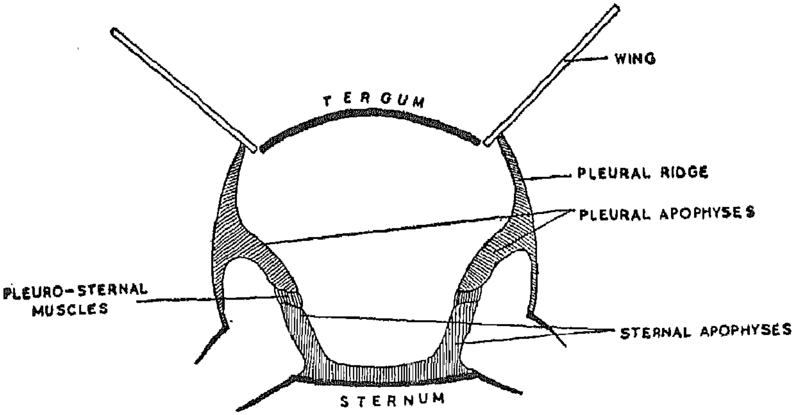


Fig. 8-10. Pleurosternal muscles.

region is more flexible, a torsion to the wing plane during motion is quite automatic. This is caused due to the changing air pressure on the two surfaces of wings when they are vibrated in a vertical direction. Thus a rotary movement of the wings is the result of anterior and posterior deflections together with the effect of changing air pressure on its heavier and membranous surfaces (Fig. 8-9 a, b, c).The rotary movement of wings are best exhibited by swift fliers that possess narrow wings such as dragonflies, wasps, bees and flies. In grasshoppers and butterflies which are provided with broad wings, the movement is mainly effected by up and down strokes.

The wing motion in flight: As already discussed, the primitive insects like Orthoptera, Neuroptera, Isoptera and Odonata move their fore and hind wings independently but in Hymenoptera, Trichoptera, many Lepidoptera and Hemiptera both pairs of wings are coupled by various means to bring about a coincidence of motion. Orthoptera and some lower Neuroptera are at a disadvantage with respect to flight as the hind pair has to work in a region of turbulence produced by the movement of the first pair. In Odonata this difficulty has been avoided as the hind wings beat first and meet the on coming air before it is disturbed by the beating of the first pair of wings. Another means of circumventing this difficulty is exemplified by *Schistocerca* (Orthoptera) where the hind pair of wings beat with a larger amplitude with the result that they become well in advance of the fore wings and thus avoid the turbulence produced by the latter.

It is known in some insects that warming up of flight muscles to about 30°C is essential prior to flight. Sphingid moths flatter their wings before taking off till the flight muscles attain a temperature of 30°C. They can, however, be made to fly straight away after keeping in an incubator at 34°C.

According to the simple aerodynamic principles the wings should create a propeller-like action in a zone of low pressure in front and above the insect for its lift and forward flight.

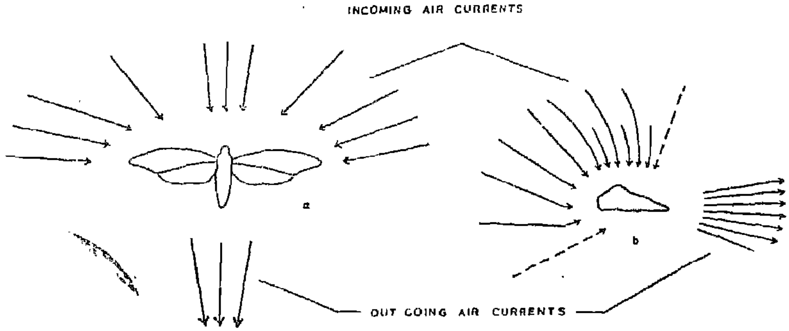


Fig. 8-11. Diagram showing the incoming and outgoing air currents which lift the insect and push it forward.

As soon as an insect starts vibrating its wings, there is at once created a zone of low pressure in front and above it. The air currents are drawn in from front, above, sides and below, the strongest currents being from the front and above (Fig. 8-11). These currents are driven away from the insects from the back in a concentrated form thereby creating a zone of increased pressure. The low pressure above the insect counteracts its weight against the pull of gravity while increased pressure behind drives the insect forward into the zone of low pressure in front. It has been calculated by workers that in large tabanids nearly two litres of air is passed per second through the wings.

Forward drive, steering, hovering, side and backward movements. The movement of wings during flight has been studied by several workers, the results being variable in different species. In general, the tips of wings during flight trace in the air an elongated figure of eight (Fig. 8-12a). This was established through high speed photography. It has been shown that motion of wings of an insect describes a series of open loops going downward and forward and from below upward and backward. Steering during flight is effected by a differential action of wings on the two sides, obviously controlled by the basalar and subalar muscles. When an insect has to turn to left or right, the plane of vibration of the wings on two sides is changed resulting in the turning of the insect in the desired direction (Fig. 8-12b).

Hovering is another common phenomenon exhibited by many insects like the syrphid flies and dragonflies when they vibrate their wings by maintaining one position in the air (Fig. 8-12c). In this type of flight, the wing vibrations are mostly in a horizontal plane and their rate is such that the zone of low pressure created above the insect is just sufficient to balance its body weight against the pull of gravity. These vibrations do not produce a zone of low pressure in front and hence there is no forward drive with the result that the insect hovers at one spot.

Certain flies, bees and specially the dragonflies are remarkable in their ability to dart suddenly to one side or move backward. The lateral movement is effected by varying the amplitude of vibrations on the two sides. When the amplitude on one side becomes so low that it almost ceases, the insect exhibits a sudden side way movement. The frequency of vibration, however, remains unaltered. When the plane of vibration is changed to such an extent that the wings actually start moving upwards in the 'down-beat', then the backward movement of the insect is effected (Fig. 8-12d).

Frequency of wing beat: The frequency with which wings beat is highly variable in insects of various groups, *e.g.*, *Apis* (Hymenoptera) 190, *Culex* (Diptera) 278–307, *Pieris* (Lepidoptera) 9, *Melolontha* (Coleoptera) 46 cycles per second. Many factors such as difference in species, age, and sex; environmental influences such as air-temperature, density, and composition and internal factors such as fatigue influence the frequency of wing beat. Many workers have attempted to measure the wing beat frequency through various techniques such as acoustic and kymographic method, high speed photography, use of microphone, amplifier and oscillograph *etc.*

It will be interesting to note that the highest value in birds *i.e.*, in humming bird is same as that found in most noctuid moths (30—50) cycles/second).

Velocity of flight: The velocity with which insect can fly varies enormously with different species. The hawk moths (Sphingidae) have been found to be the swiftest fliers that can fly at a velocity of 15 metres/second. A tabanid fly can go at a rate of 14 metres/second whereas the speed of a dragonfly varies from 4–10 metres/second. A housefly travels from 2 to 2.3 metres/second and a bumble bee 3 to 5 metres in one second. An unladen honey bee can fly at a rate of 3.7 metres/second but when loaded with pollen, it can hardly go at a speed of 2.5 metres/second. Among the good fliers the sphingids, many butterflies and locusts are capable of travelling hundreds of kilometres without a break.

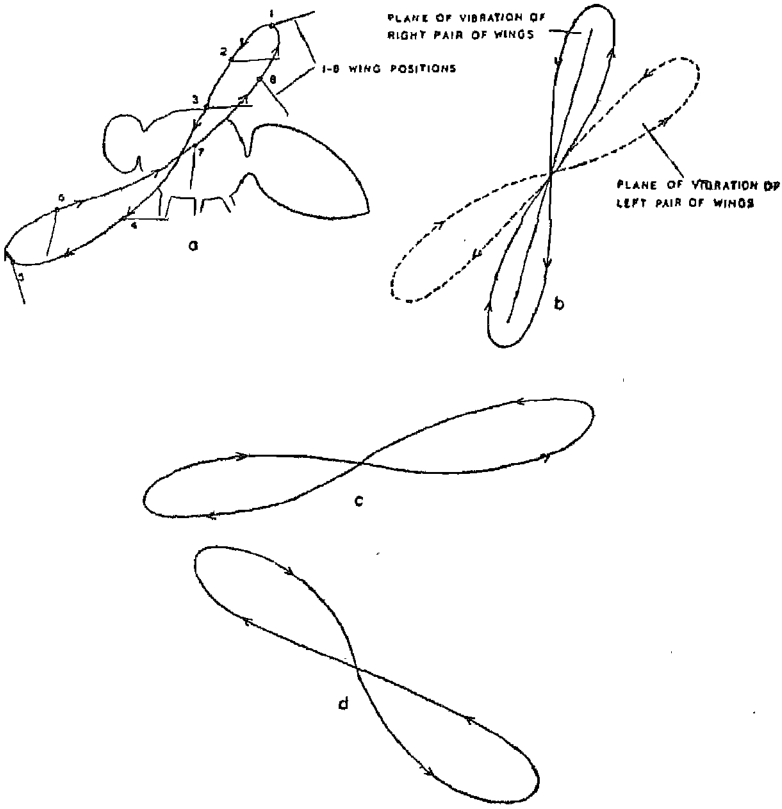

PLANE OF VIBRATION OF RIGHT PAIR OF WINGS
1-8 WING POSITIONS
PLANE OF VIBRATION OF LEFT PAIR OF WINGS
1
2
3
4
5
6
7
8
a
b
c
d

Fig. 8-12. Diagrammatic representation of different planes of wing vibration., (a) forward flight (b) turning to right (c) hovering (d) backward flight.

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9

THE DIGESTIVE SYSTEM AND DIGESTION IN INSECTS

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A. The organs of digestion

Insects can feed on an unusually wide variety of foodstuffs. The organs primarily concerned with the intake of food are the *mouth parts* which are variously modified to suit the type of food required to be taken by the insect. Thus, the feeding organs in various insects are specialized for obtaining particular kind of food so much so that the study of mouth parts itself constitutes an important branch of entomological studies.

The food, after having been obtained in one way or the other, has to pass through the digestive tube which extends from mouth to anus and is known as the alimentary canal. Length of the alimentary canal varies and in some insects it may be several times the body length. Normally, in phytophagous forms alimentary canal is longer than that in carnivorous insects. However this general rule may not always hold good. All insects take their food into the alimentary canal except the earliest stages of some parasitic insects which absorb nutrients through the general body surface.

The digestive system includes food tract and its accessories like salivary glands and gastric caeca. Malpighian tubules though connected with the digestive system, are excretory organs, hence these would be dealt under excretory system. The alimentary canal (Fig. 9-1) is an asymmetrical tube and is divided into three main parts *viz.*, the foregut or *stomodaeum*, the midgut or *mesenteron* and the hindgut or *proctodaeum*.

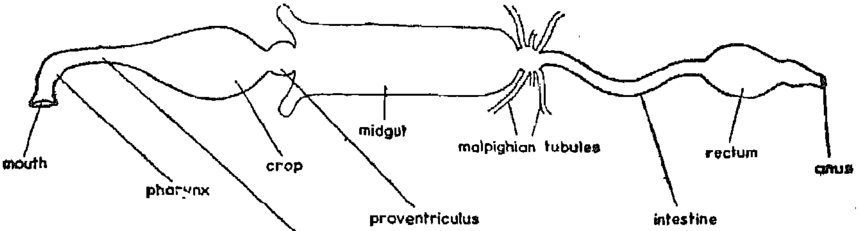

Fig. 9-1. Diagrammatic representation of alimentary canal and its associated parts in an insect (after Wigglesworth, 1970).

The foregut or stomodaeum: It is the anterior part of the alimentary canal and is ectodermal in origin. It is generally divided into pharynx, oesophagus, crop and proventriculus. *Pharynx* is the anterior most portion of the foregut, continuous with the mouth opening. It has additional dilator muscles which are very well developed in sucking insects like Hemiptera and adult Lepidoptera as in these groups pharyngeal pump is used to take in fluid diet from the host. In insects with biting and chewing mouth parts, pharyngeal muscles help in pushing the food from mouth to oesophagus. Pharynx is followed by an undifferentiated tube of variable length known as *oesophagus*. Its function is to receive food from the pharynx and pass it onwards to the crop. *Crop* (Fig. 9-2-ii) is the dilation of the foregut and is meant for storing food. Generally it is a dilated posterior portion of the oesophagus but in some fluid-feeding insects *e.g.*, in *Lucilia* larvae it is in the form of a lateral diverticulum (Fig. 9-2-iii). When empty, the crop in most insects remains in a collapsed state but in others like cockroach, it is kept distended by the air swallowed in by the insect. Usually there is no secretory or digestive activity in this region. However, some digestion of food may take place because of the mixing of enzymes from the salivary glands reaching the crop along with food. The enzymes are also regurgitated from the midgut to crop. Although the proventriculus prevents the passage of solid food from midgut back to foregut, it permits regurgitation of fluids. *Proventriculus* (gizzard) (Fig. 9-1) is the last part of the foregut joining the midgut. Its main function is to control the passage of food to midgut as well as to prevent regurgitation of food from midgut back to the foregut. It is modified in different ways to suit the requirements of insects with varying feeding habits. It is very much reduced in fluid feeders and is represented just by a simple valve at the origin of the midgut. In cockroaches, crickets and grasshoppers proventriculus has six strong chitinous plates or teeth which grind the food. In case of honey bee the proventriculus is an anterior invagination into the crop and ends in four mobile lips, each armed with a number of spines. In addition to controlling the movement of food, it is able to separate pollen from nectar in the crop. By the movement of the crop the pollen is kept dispersed while the lips of the proventriculus make snapping movements in such a way that the spines strain off the grains of pollen and retain them. In this way a bolus of pollen is formed and is allowed to pass into midgut and nectar is retained in the crop for regurgitation and further processing to form honey.

The midgut, mesenteron or ventriculus: It is endodermal in origin. This part of the alimentary canal (Fig. 9-1) is concerned primarily with the secretion of enzyme, digestion and absorption of food. Midgut cells also play some part in excretion as in *Rhodnius* which feeds on vertebrate blood; the haemoglobin is broken down intracellularly to haematin and biliverdin which are excreted into the lumen from where these products find their way to out side.

In many insects internal surface area of the midgut is increased by the development of varying number (usually 2-6) of sac-like diverticula known as *gastric* or *enteric caeca*. These are situated at the beginning of the midgut. However, in cockroaches, mantids, and larval Culicidae these are eight in number, while in the larvae of Scarabaeidae these are numerous and arranged in three annular series. Among various predacious Coleoptera caeca are represented by numerous villiform processes and in Collembola and Lepidoptera these are generally wanting.

Midgut is a simple undifferentiated tube but in some Diptera it is divided into an anterior, cardiac and a posterior, ventricular part. In some Heteroptera it is imperfectly or sharply distinguishable into four parts which are often termed as ventriculus i to iv. The fourth ventriculus in some species possesses mycetomal caeca harbouring bacterial symbiotes. In many plant-feeding hemipterans which have to imbibe large quantities of fluid to obtain adequate nourishment, the midgut is structurally and functionally modified for rapid elimination of excess of water from the food. Excessive dilution of haemolymph is thus avoided and at the same time the food is concentrated to facilitate enzyme activity in the gut. Such type of adaptation, is not found in nectar feeding adults of Lepidoptera (Fig. 9-2-iv), Hymenoptera and Diptera because their nutritional requirement is fulfilled by imbibing a small quantity of nectar which they need just for maintenance, as their growth is already complete and the reserves built up during larval life are enough for the development of eggs. However, these insects store the nectar in the crop lined with impermeable cuticle and from there small quantities are passed into midgut when required and thus dilution of haemolymph is probably minimised.

In plant sucking Pentatomorpha the caeca of the fourth ventriculus remove water from the haemolymph and there is a constriction between third and fourth ventriculus which ensures the backward flow of water to the rectum. In blood sucking insects the amount of fluid taken is not large and blood corpuscles contain sufficient nutriment; the fluid contents can be eliminated rapidly with little loss of nutriments for the insect. In tsetse fly *Glossina*, water is removed from the blood in the anterior half of the midgut and very quickly eliminated via the Malpighian tubules. In the diaspine coccids the midgut is a closed sac, entirely disconnected from the hind intestine, and the digestive juices render the food contents capable of passing by osmosis into the main haemo-

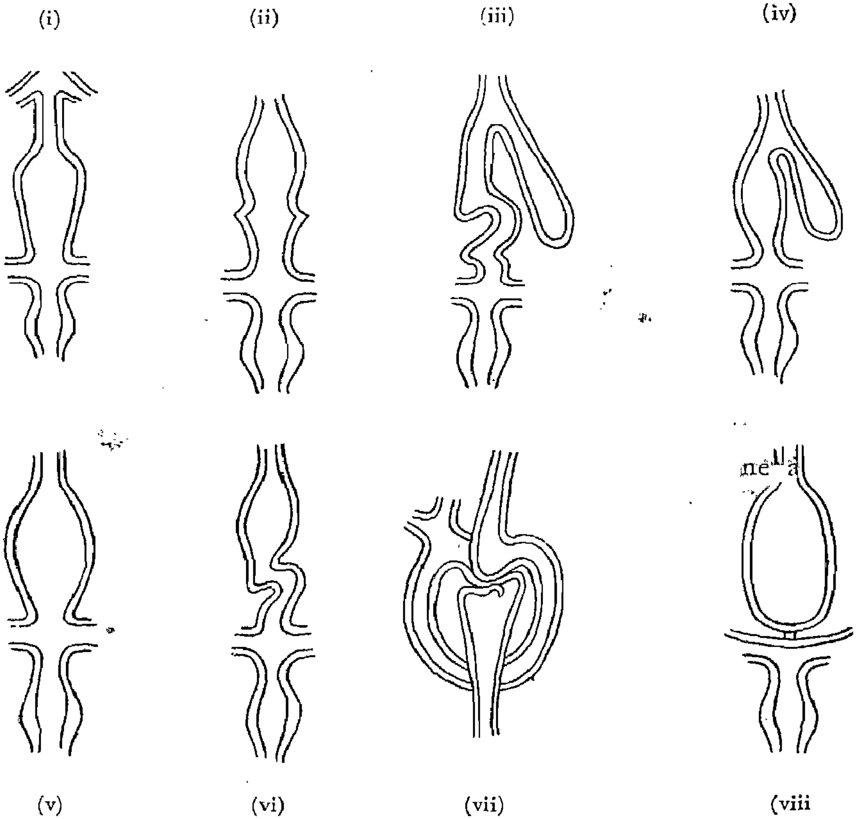

Fig. 9-2. Diagrammatic representation of different types of digestive organs occurring in insects. (i) Primitive type (caterpillars) (ii) Orthoptera (iii) higher Diptera (iv) simple Diptera (Nematocera) and adult Lepidoptera (v) Siphanoptera (vi) Hemiptera (vii) Homoptera (viii) larvae of Hymenoptera and Neuroptera (After Wigglesworth, 1970).

coelic cavity. The waste substances there from are then taken up by the greatly enlarged Malpighian tubules.

Among many homopterans (Fig. 9-2-vii) (membracids, coccids, psyllids, cercopids *etc.,*) the terminal region of the midgut is in intimate contact with the posterior part of the midgut or anterior part of hindgut forming a *filter chamber.* By this arrangement the excess water of the plant-sap is able to pass directly from the anterior part of the midgut to the last part of the midgut without diluting the haemolymph. In Cicadoidea, filter chamber is formed by the association of anterior part of midgut and hindgut while in cercopids the posterior midgut along with Malpighian tubules is invaginated to a diverticulum formed by the oesophagus. The transfer of fluid in the filter chamber from anterior to the posterior part of intestine in not a mere passive filtration but is an active process in which the epithelial cells absorb water and actively transport to the other chamber.

Unlike the fore and hindgut the epithelial cells of midgut do not have intimal lining, with the result that the cells are likely to be injured during the passage of solid food through this part of intestine. In many insects a thin cuticular lining is secreted periodically from the entire surface of epithelium and food particles remain enclosed in this membrane. In other insects the membrane is secreted from special glands located at the junction of fore and midgut. This protective cuticular lining is termed as a *peritrophic membrane* which logically is expected to have no function to perform in fluid feeders. There are however several exceptions. It is absent in ants and larvae of *Anthrenus,* which feed on solid food while it is present in adult *Pieris* and adults of other Lepidoptera which are essentially nectar-feeders. In carnivorous carabids, the membrane is absent because the regenerative cells are extremely active and are able to repair the epithelial lining several times during the feeding activity of insects. Moreover the secretion of digestive enzyme in this insect is of *holocrine* type in which the midgut cells break down completely during their secretive phase. In spite of its cuticular nature the peritrophic membrane is permeable to enzymes and products of digestion.

The hindgut or proctodaeum: Like the foregut this region of the intestine is ectodermal in origin and is lined with cuticular intima which is comparatively thinner and more permeable than that of the foregut. The muscles are poorly developed except around rectum. The anterior part of hindgut (Fig. 9-1) is often called *pylorus* possessing pyloric valve at the junction of midgut. Externally the pylorus is marked by the presence of insertion point of Malpighian tubules. Pylorus opens in an undifferentiated chamber called *ileum* which, in wood-eating termites and beetles is greatly enlarged to form a pouch containing protozoan symbiotes which help in digestion of cellulose. In these insects the hindgut is the main seat of digestion. In blowfly larvae the epithelial cells of ileum excrete nitrogenous waste matter in the form of ammonia. The posterior portion of hindgut is called *rectum* which is generally a thin-walled chamber containing variable number of muscular rectal pads which play important role in reabsorption of water, essential ions and amino acids from the urine. During the excretory activity of Malpighian tubules these essential substances are removed from haemolymph and therefore are retrieved by the insects. In certain aquatic insects the rectal walls possess rectal gills. By the movement of rectum water is taken in and pushed out with the result that fresh water comes in contact with the gills to bring about effective respiration. Forcible ejection of water from rectum is used as a mechanism by aquatic forms for rapid forward movements.

Salivary glands: These are in fact labial glands and are situated in the thoracic region on either side of foregut. Their structure is very variable in different groups of insects. The secretion from the two salivary glands is poured out at the base of hypopharynx through salivary ducts. In majority of insects their function is to moisten the food and to digest starch with the help of enzymes contained in their secretion. In silkworms, the salivary glands are modified into silk glands which secrete fibrinogen which changes into elastic protein fibrinogen and sericin. The fibrinogen changes into an elastic protein, fibroin which along with sericin forms silk. The terminal end of salivary duct is modified into a press which spins the silk into thread where fibroin forms the central core covered with the layer of sericin. In male *Panorpa* the greatly enlarged salivary glands have different function of secreting a mass of material which is offered to the female during copulation.

B. Digestion

Before the process of digestion starts insect has to find food and feed upon it. In some insects finding food has little problem as they are surrounded by an abundance of food from the time of hatching because of the ovipositional habits of the female. In phytophagous forms the female oviposits on or near the food plant on which young ones feed. Similarly amongst the saprophagous and parasitic insects the female selects appropriate substrate or host for oviposition so that larvae have an assured supply of proper food. The larvae of social insects like honey bees are given food by the workers or the parents. Insects have a capacity to recognise and to get attracted towards food suitable for them or for their progeny from a distance mainly as a result of visual response. Olfactory responses also play important part especially when insects are near or on the food. The predators obtain their food either by waiting for the prey as in mantid or by actively pursuing as in adult Odonata.

Specificity of insects to particular plants or plant families varies widely. No particular plant family can normally be considered to be attacked exclusively by a particular species or a group of insects. Even those insects which can broadly speaking be called polyphagous (*e.g., Schistocerca*) also exercise some degree of choice. Even those which generally feed on plants of a particular plant family, may exercise a choice within members of the same family. Larvae of *Athalia lugens proxima* (Klug) which are pests of cruciferous crops mainly in the nursery, when given a choice between radish (*Raphanus sativus*), turnip (*Brassica rapa*), mustard (*B. nigra*), 'rai' (*B. juncea*), cauliflower (*B. oleracea* var. *botritis*), *cabbage* (*B. oleracea* var. *bullata*), and knol khol (*B. oleracea* var. *gongylodes*) prefer turnip if all the plants are of the same age. Insects like *Bombyx mori* feed exclusively on mulberry and *Schoenobius incertulas* on paddy. These forms are often termed as monophagous.

Food and feeding habits bear a direct relation to the morphology and physiology of insects in which digestive system and mouth parts have undergone adaptive modification. In larval and adult stages of the same species in Lepidoptera the food, mouth parts, alimentary canal and digestive enzymes vary because of different feeding habits. This also eliminates chances of competition amongst different feeding stages of the same species and thus brightens the chances of their survival.

The digestive system is mainly concerned with changing the food in an assimilable form with the help of a large number of enzymes secreted by epithelial cells of its lining and associated glands. The digested products are absorbed by special cells and transferred to the haemolymph. In special cases, as in termites and wood-boring beetles, the process of digestion is supplemented by the microorganisms of the gut. These insects feed on cellulose but lack in the source of the enzyme, *cellulase* which is provided by the microorganisms. As already stated elsewhere, most of the enzymes are secreted by the midgut which is the main seat of digestion and absorption. The salivary glands in many insects secrete enzymes which hydrolyse starch. For the proper functioning of enzymes pH in the gut is maintained at optimal levels in different parts of the alimentary canal. During the secretory activity the epithelial cells undergo various changes. In some insects, the secretions are poured out from the free margin of the cell while in others, the cells burst to release the enzyme, the former mode of secretion is called merocrine and latter holocrine.

In rare cases certain amount of digestion is carried out in the food itself where the insect regurgitates gut enzymes into the substrate. This is called *extra-intestinal* digestion. The larvae of *Dytiscus* regurgitate the gut contents into the body of the prey which gets completely paralysed and at the same time its tissues start disintegrating due to the action of enzyme. This digested food is then sucked by the predator.

pH of alimentary canal: The pH has a great influence on the activity of digestive enzymes and on the absorption of food. The pH of the midgut varies from 6 to 8. However, in the larvae of Lepidoptera and Trichoptera it ranges from 8 to 10. Generally in foliage and wood-eating species pH is slightly alkaline but in predacious forms pH is weakly acidic. pH of midgut of clothes moth is 9.9 and that of wax moth 8.4. Both wool and wax are more readily degraded under alkaline conditions. The pH of the larval midgut of *Stathmopoda melanochra* and *Titanoceros thermoptera* is 8.3 to 8.6 and 8.4 to 8.6 respectively. The gut has a buffer system by which it resists major changes in pH due to the nature of food. This buffering capacity of the gut increases in the starving insects by special secretions of unknown nature.

Movement of food in the gut: Once the food is taken in, it has to pass through the entire length of the alimentary canal with such a rate that the digestion and absorption take place at proper sites. The food is retained in some regions for much longer time as in crop which acts as a temporary reservoir in insects like cockroach to permit mechanical processing by gizzard. The intestine generally shows three types of movements peristaltic, antiperistaltic and gross contractions. The former two types cause forwards and backward movements respectively causing agitation of the food to ensure proper mixing of digestive juices. At certain times, one or the other type of movement may predominate to push the gut contents only in one direction. The movements of different parts of intestine can be independent of each other and are not necessarily synchronised.

Food may remain for considerable time in certain parts of the gut. Adult flies can retain part of the meal in the crop for several days. In blood-sucking gnats the food reaches midgut in thirty minutes but takes nearly six hours to reach the rectum. In cockroach food takes nearly six hours to reach the hinder part of intestine. The rate of passage of food often increases if poisonous chemicals are mixed with the diet. The nervous stimulus and state of starvation also affect this rate.

Enzymes: Enzymes are highly specific biological catalysts which initiate and accelerate the reaction. These are either proteins or compounds in which the protein molecules are the main components. In some cases non-protein groups are attached to enzyme molecules to make the catalyst active. These groups are called *prosthetic* groups. Often the non-protein groups exist independently and their presence is essential for the activity of the enzyme. These are called *co-enzymes*. Enzymes may therefore be defined as water soluble colloidal organic catalyst more or less specific in action produced by living cells and destroyed by heat.

Enzymes accelerate chemical reactions by (i) hydrolysis and its reverse process, dehydration and (ii) oxidation and its reverse process reduction. Almost all digestive enzymes are hydrolases (causing hydrolysis). A disaccharide in the intestine, is broken down into two glucose molecules, whereas molecule of water reacts under the influence of the enzyme. This addition of water molecule is a hydrolytic process. The other type of reaction is oxidative and is carried out by respiratory enzymes. As happens in the living cells carbohydrates combine with oxygen through a series of reactions involving different groups of enzymes called *desmolases*, producing carbon dioxide, water and energy. This energy reaction is caused by breaking the C-C- bonds of the food material (see chapter 11).

All the above reactions occur at the body temperature only due to the enzymes, but if such reactions are to be brought about artificially outside the body and without the aid of biological catalysts, one would need very high temperatures or concentrated acid-conditions which no living system can withstand.

Different types of enzymes have been isolated from insect gut. These are (i) *carbohydrases acting upon disaccharides* and polysaccharides including starch (ii) *proteases* acting upon proteins and (iii) *lipases* on fats and oils. In a general way, it can be said that the types of enzymes present in the gut have some correlation with the nature of food. An omnivorous insect like cockroach possesses protease, lipase, amylase, invertase and maltase, hydrolysing proteins, lipids, starch, cane sugar and maltose respectively. Salivary glands secrete amylase and maltase which can be detected even from the content of the foregut. In the gastric caeca presence of lipase, protease and invertase has been shown in some orthopterous insects. The midgut content shows the largest number of enzymes many of which are secreted by its epithelial layer. These are mainly invertase, lipase and protease. The presence of amylase and maltase in the midgut of some groups of insects could be due to their own secretions or may be contributed by the salivary glands. In the hindgut several enzymes secreted by other parts of intestine can be still detected in the gut contents. As a rule the hind-gut epithelium does not secrete digestive enzymes.

It has already been stated that the enzyme system of insect is adapted to its feeding habits. In carnivorous insects protease and lipase are more predominating. In carabids and *Glossina* (blood-sucking tsetse fly) proteases are extremely active and carbohydrases are practically absent except a very weak amylase in the midgut. In *Chrysops* (Tabanidae) which feed on blood and nectar both, the midgut secretes fairly active invertase and proteolytic enzymes.

Very often Malpighian tubules have been reported to contain varying quantities of peptidases. It is still doubtful whether these enzymes play any role in the digestion of food. Several workers feel that these are the intracellular enzymes taking part in cellular metabolism only. If mosquito larva is fed upon olive oil, the latter enters the Malpighian tubules, where it is acted upon by the lipase showing thereby the possibility, although remote, of the digestive function of enzymes originating from Malpighian tubules.

Insects are unique in having some of the members which are capable of utilizing odd substances like wool, hair, feathers and cellulose. The cellulose is digested by the termites with the help of symbiotic protozoans which are harboured in the hindgut. Hair and feather contain a protein called keratine which is resistant to proteolytic enzymes due to the presence of varying amount of cystine, the sulphur of which forms disulphide bond between two adjacent polypeptide chains. Among animals only insects like Mallophaga, dermestids and clothes moth are able to digest keratine due to the presence of a special mechanism including high alkaline conditions (pH 10) in the gut which renders the disulphide bridge of the molecule less stable, so that the proteolytic enzyme can effectively bring about hydrolysis. The capacity of wax moth larva to digest bees wax to some extent is probably due only to bacterial symbiotes located in the gut lumen.

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10

RESPIRATORY SYSTEM

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Respiration is a process of interchange of gases between the environment and the blood or cellular tissues of the organism. This includes both chemical and physical phases. The chemical phase is concerned with oxidation in the body tissues resulting in the formation of carbon dioxide and water. The physical phase involves transportation of air to tissues and elimination of carbon dioxide. The special mechanical devices that are developed to facilitate the respiration in animals constitute the respiratory system.

The great majority of insects breathe by means of tracheal tubes, which usually open at the surface of the body through a number of openings and convey air directly to the tissues. This system of tubes is known as tracheal system.

Rarely some insects have imperfectly developed system or no trachea, where the integument acts as a respiratory organ. Besides this, in immature stages of many aquatic insects respiratory organs are present which are known as gills or branchiae.

TRACHEAL RESPIRATION

It is a system of open tubes called *trachea* permitting direct contact of air with the tissues through a number of openings known as *spiracles*. Trachea are elastic tubes which when filled with air present a silvery appearance. Histologically these are similar to general body wall. Ectoderm (Fig. 10-1) invaginates along the sides of the body, the external orifices of the depressions become the openings known as spiracles; the internal tubular parts extend inwards and branch into ramifying tubes that eventually extend to all parts of the body of insects.

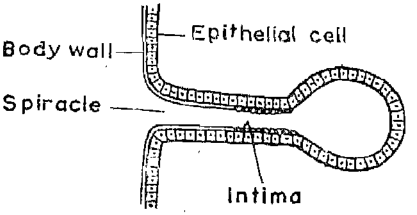

Fig. 10-1. Diagrammatic representation showing the origin of a trachea.

The trachea consists of a layer of flat epithelial cells (*ectotrachea*) which secretes a cuticular substance called the intima (*endotrachea*). The surface of the intima is thickened

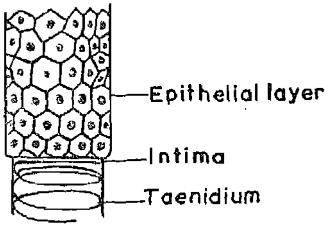

Fig. 10-2. Histological structure of a trachea.

and ensures that it remains round and opens even under conditions of bending pressure (Fig. 10-2). The tracheae divide and redivide, becoming smaller and smaller; finally each ends in a cluster of minute branches, *the tracheoles.* The tracheoles are less than 2 μ in diameter; these possess taenidia (visible only under electron microscope) but no regular layer of epithelial cells. The base of each cluster of tracheoles has a web-like cell known as *end cell* or *tracheole cell* with extremely thin protoplasmic extensions. These extensions appear to surround and follow the tracheoles. The tips of tracheoles lie alongside, between and actually within the tissue cells of the body (Fig. 10-3). It is believed that most of the respiratory gas-exchange in the tissues occurs through these tracheole tips.

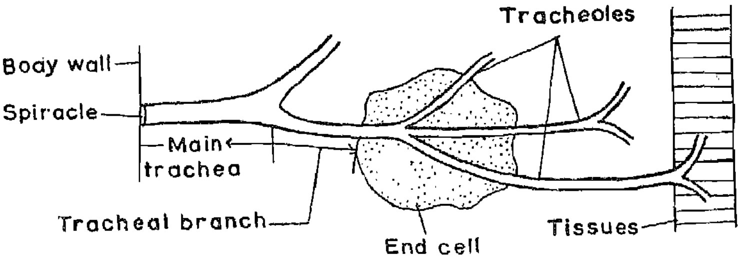

Fig. 10-3. An open trachea of a generalized insect.

The earlier definition of tracheoles *i.e.,* fine branches lacking taenidia or branches of the tracheae within the end cells is no longer acceptable. Taenidia are present throughout and permeability of tracheae and tracheoles are similar. The end cells play important role in the formation of tracheoles, but their origin and function are still not certain. Unlike epidermis both tracheal cells and tracheae are permeable to gases but tracheae are impermeable to liquids.

Organization of tracheal system attains a definite fundamental pattern in higher groups of insects. In primitive insects, the tracheae are not anastomosing (Fig. 10-4). This condition is found in Campodeidae (Diplura) and Machilidae (Thysanura). In highly developed insects, there is well-developed system of the fundamental pattern by uniting some of the branches from consecutive and opposite spiracles. There are three main trunks, *lateral longitudinal, dorsal longitudinal* connected with the lateral (Fig. 10-5) by palisade tracheae, and less frequently with *ventral longitudinal* trunks. Transverse, dorsal or ventral commissures connect the systems of each side.

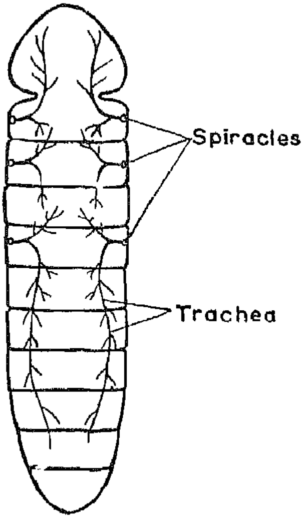

Fig. 10-4. A primitive tracheal system.

The dorsal longitudinal trunks give off segmental branches which pass to the heart and dorsal musculature. Visceral branches, which supply the digestive canal and reproductive organs take their origin from the palisade tracheae or directly from the spiracular longitudinal trunks in the thoracic region, and the basal tracheae of the developing wings usually take their origin in close association with those of the legs of the meso- and metathorax. The head and mouth parts are principally supplied by branches derived from the anterior most spiracle and the dorsal longitudinal trunks.

Spiracles

These are morphologically the mouths of the ectodermal invaginations which give rise to tracheal system as described earlier. These are normally placed on the pleura of the thoracic and abdominal segments, but their exact position is very variable. In the abdomen of most insects these are seen to lie in the soft membrane between the terga and sterna, sometimes towards the front or back of their segments. In many insects, particularly on the thorax, these occupy an intersegmental position, being situated just in front of each segment, to which these are generally referred to or these may be no longer situated on the pleura but come to lie on terga, near the side margins of the latter as in *Apis* and *Musca*.

According to number and arrangement of functional spiracles respiratory system is of three types:

Holopneustic: This type has ten pairs of spiracles, two thoracic, one on metathorax and the other on pro- or meso-

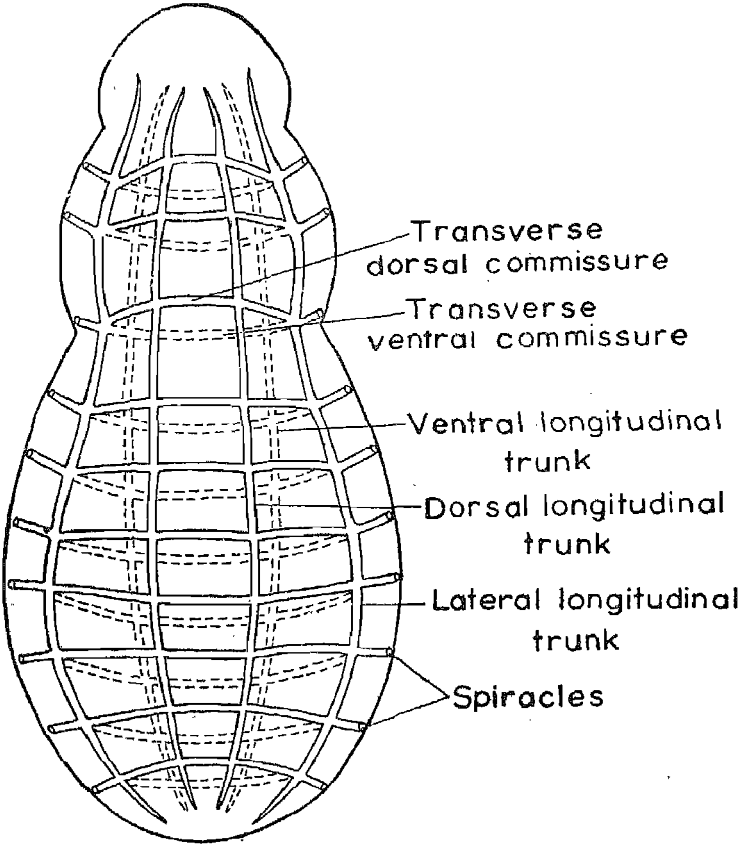
Transverse dorsal commissure
Transverse ventral commissure
Ventral longitudinal trunk
Dorsal longitudinal trunk
Lateral longitudinal trunk
Spiracles

Fig. 10-5. Well-developed tracheal system in an insect.

thorax and eight on first eight abdominal segments *e.g.*, Diptera (Bibionidae) and Hymenoptera.

Hemipneustic: All the ten pairs are not functional, one or two become non-functional. It has the following four subdivisions:

Peripneustic—Prothoracic and abdominal spiracles are open, metathoracic being closed; only nine spiracles are open *e.g.*, terrestrial larvae of Neuroptera, Mecoptera, Lepidoptera, many Hymenoptera and many Coleoptera.

Amphipneustic—Only prothoracic and posterior abdominal spiracles are open, *e.g.*, larval Diptera.

Propneustic—Only one pair of spiracles is open on prothorax *e.g.,* dipteran pupae.

Metapneustic—Only one pair of abdominal spiracles is open *e.g.,* some larval instars of Diptera.

Apneustic : Here none of the spiracles are functional *e.g.,* Collembola, Protura, parasitic Diptera and immature stages of endoparasitic Hymenoptera. Air enters the closed tracheal system by diffusion through the general body surface or specialized extensions of it such as gills or branchiae.

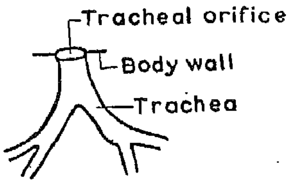

Fig. 10-6. Simple type of spiracle.

Structure of the spiracle varies in different insects. In simple type (Fig. 10-6) the spiracles are simple, opening from integument into the tracheae. There is no provision for regulating the size of the spiracle openings, *e.g.,* Colembola (*Sminthurus*) and thoracic spiracles of plecoptera. In the *atriate type* tracheal aperture sinks into secondary depression of the integument known as atrium or *vestibule*. Atrial walls are often clothed with hairs and other cuticular processes called *trabeculae* (Fig. 10-7) which act as filters. The atrial orifice may be produced to form lip-like projections or valves which may be movable by special muscle attachments.

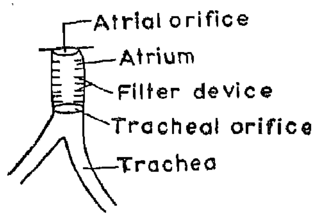

Fig. 10-7. Atriate type of spiracle.

The atriate type of spiracles possess "closing apparatus" though it serves for both opening and closing. There are two types of closing mechanism as described below:

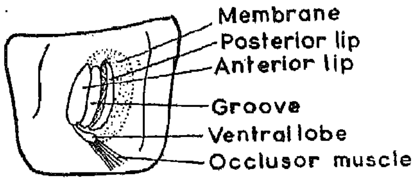

Fig. 10-8. Atriate type of spiracle with external closing apparatus.

External closing apparatus : This is very common in many insects such as Hemiptera, Elateridae and grasshoppers.

There are two movable lips, anterior and posterior which are united by ventral lobes. These open by means of their own elasticity but close by an occlusor muscles which arise from a process in mesocoxal cavity (Fig. 10-8).

In case of some Hymenoptera, first thoracic spiracle is closed by operculum, a large flattened lid like anterior lip of atrium.

Internal closing apparatus: It is very commonly seen in abdominal spiracles of most insects. Here, atrium is a well developed open cavity of the integument and may be long or tubular. Atrial lips are not developed or if developed these are not functioning as closing apparatus, but entrance is however guarded by a filter apparatus, usually in the form of two rows of *matted* brushes projecting from opposite walls of atrium (Fig. 10-9). There are two common subtypes:

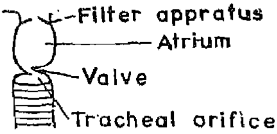

Fig. 10-9. Atriate type of spiracle with internal closing apparatus.

(i) *Pinchcock apparatus*—It consists of two sclerotic bars in opposite walls of atrium just before the mouth of trachea with a occlusor muscle stretched between their projecting ends (Fig. 10-10). This brings about the closing of the entrance of trachea. Usually a second muscle arising on the body wall is inserted on the end of the anterior bar opposite the attachment of the occlusor muscle and acts as a dilator of spiracle. It is found mostly in hemimetabolous insects.

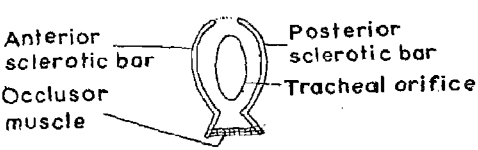

Fig. 10-10. Atriate type of spiracle with pinchcock apparatus.

(ii) *Single-wall closing apparatus*—It consists of crescentric elastic bar or "bow" whose ends are produced outside the atrium and an occlusor muscle is stretched in between. It has got a closing band which is a soft convex fold projecting in the lumen of atrium opposite to bow. This type opens by the elasticity of the bow, is usually modified in various ways and is provided with dilator muscle. It is common in holometabolous insects.

Spiracles are hydrophobic *i.e.,* their surface resists entry of water. The hydrophobic condition is created by oily substance which is secreted by peristigmatic glands situated close to spiracles.

In many winged-insects the tracheae are dilated in various parts of the body to form thin-walled vesicles or air-sacs. These are extremely delicate structures and usually lack taenidia. In cockchafer beetles (*Melolontha*) and in grasshopper, air sacs are dilations of smaller tracheae. In *Musca* and other Diptera main trunks dilate to form massive air sacs specially in abdomen. Air sacs are also found in Lepidoptera and Odonata. These are mainly developed in swift flying insects and help them in lowering of specific gravity which makes easier the flight of large species. The main function of these air sacs is to give an increased ventilation *i.e.*, large amount of air can be inspired and expired, during respiratory movements.

INTEGUMENTAL RESPIRATION

In insects that do not have tracheae, or have an imperfect or a secondarily closed tracheal system and are not provided with other devices for respiration, the exchange of gases takes place directly through the integument. The best known examples of insects having this type of respiration are majority of Collembola, Protura, aquatic Chironomidae and parasitic insect larvae which live entirely submerged in the liquids or tissues of the hosts.

Though the majority of free living insects have well-developed tracheal system, expiration of carbon dioxide does take place partly through the integument.

GILLS OR BRANCHIAL RESPIRATION

(i) **Tracheal gills :** These are present in majority of aquatic larvae and are usually borne on the abdomen. These are filiform or more or less lamellate organs which are well supplied with tracheae and tracheoles. (Fig. 10-11). In many cases these are only organs of respiration but in others these are accessory in function and co-exist with open spiracles. In general, a good deal of gaseous exchange takes place through these structures.

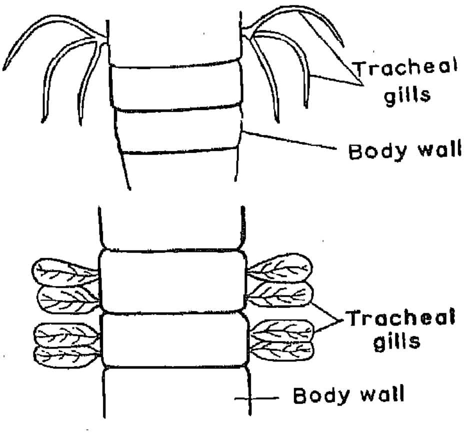

Fig. 10-11. Different type of tracheal gills.

(ii) **Spiracular gills:** These occur in pupae of some beetles and dipterous families, and are extensions of spiracles or the cuticle surrounding them to form long, partly hollow processes. One or more pairs of spiracles usually form spiracular gills. These structures are adapted both for aquatic and aerial respiration. In water, the film of air which these support presents a large gas-water interface for diffusion, while in air the interstices of gill provide a direct route for entry of oxygen and water loss is limited because the gill opens into atrium of the spiracle. Thus in air, water loss through the spiracles would be little or no greater than in terrestrial insects.

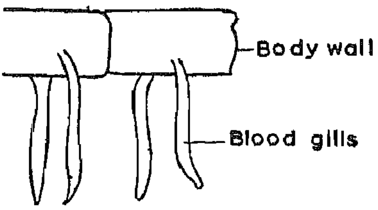

Fig. 10-12. Blood gills.

(iii) **Blood gill** : These are found in larval Trichoptera and Diptera and are well developed in the larvae of *Chironomus* (Diptera) (Fig. 10-12). These are tubular or digitiform and are sometimes eversible. These derive their name from the fact that these contain blood but not as a rule tracheae, although occasional tracheoles may be present. These structures help in the absorption of water and inorganic ions rather than respiration.

(4) **Gaseous plastron:** In some aquatic forms of the family Donacinae (Coleoptera) and some of the Hemiptera, there is a permanent thin layer of gas known as gaseous plastron held by various means around the body surface. These insects never visit the surface throughout their life history, because the volume of plastron (airfilm) remains constant and is maintained in its position by various hydrofuge devices like hairs which are spaced so closely together that water under normal pressure cannot enter these. The spiracles are situated just below the plastron layer but the air itself does not enter the tracheae. The plastron acts as a physical gill through which the dissolved oxygen from the water enters the plastron and then diffuses into insect system. Plastron type of respiration is found in many insect eggs and pupae of aquatic insects.

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11

THE CELL AND CELLULAR RESPIRATION

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THE primordial organism was unicellular which during the course of evolution had undergone structural and functional modifications giving rise to multicellular organisms with specialized tissues and organs. In spite of the specialization and division of labour the cell remained the essential unit where active life-processes are carried out. The activity of body organs and hence the body as a whole, represents the summation of the processes undergoing in cells. The basic unit of all living things is still the cell as it was in the past when life originated. In multicellular animals including insects all cells are not alike and differ in structure and function. Even the same cell keeps changing from moment to moment because new materials enter it and waste products leave it continuously; protoplasm undergoes physical and chemical transformations. These phenomena in the cell are controlled by a delicate yet highly complicated set of conditions like osmosis, diffusion, permeability of membrane, colloidal system and chemical activity for the production of energy needed for the life processes *etc.*

In spite of cellular differences, all cells have certain basic functional uniformity. We shall see mysteries of cells in a generalized way in this chapter.

THE CELL

Morphology of cell

Protoplasm or protoplast: It refers to the "living" substance inside the cell (Fig. 11-1) and includes in it a number of small bodies. Protoplasmic substance minus nucleus is often referred to as *cytoplasm.* The protoplasm consists of 80% water and rest are carbohydrates, proteins, fats and pigments. All these may serve as fuel for generating energy during cellular respiration.

Cell membrane or plasmalemma: The outermost layer of protoplasm has a double-layered living cell membrane through which food, waste product and other substances have to pass in and out. The membrane at several points invaginates deep into the cytoplasm and is profusely branched to form a vast system of *endoplasmic reticulum.* The membrane is elastic and *differentially permeable.* It consists of proteins and lipids, and is about $75\mathrm{A}^\circ$ thick.

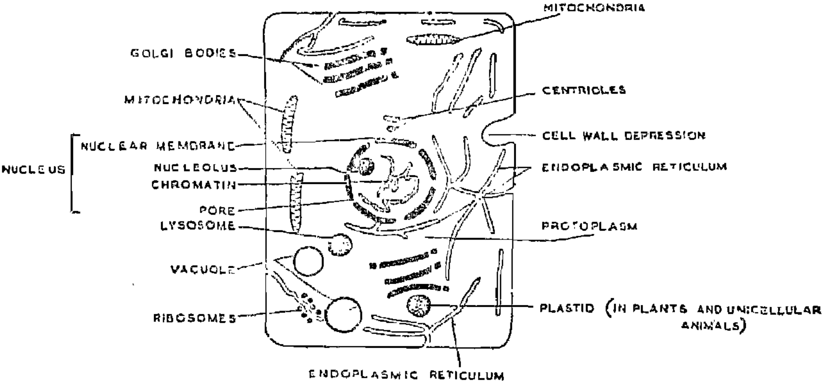


Fig. 11-1. Internal structure of a typical cell according to the modern concept based upon electron microscopic investigations.

Endoplasmic reticulum: As stated above, the cell membrane invaginates into the cytoplasm to form endoplasmic reticulum and thus increases the area of cell surface. The vesicles of reticulum are not continuous with cytoplasm. These form the membranes of nucleus and Golgi bodies. Reticulum is in close contact with *ribosomes* suggesting that protein synthesis takes place at these points as ribosomes contain 90% of the cell RNA.

Golgi bodies : These are canalicular in appearance like endoplasmic reticulum. No ribosomes are attached to the surface which suggests that Golgi bodies are not the site for protein synthesis. Presence of lipid globules and protein granules indicates that Golgi bodies may have a storage function.

Ribosomes: These are particles of about $200\ \mathrm{A}^\circ$ size and are found either attached to endoplasmic reticulum or lying freely in the cytoplasm and under both the conditions these take part in protein synthesis from amino acids present in the cell. The nucleic acid from nucleus provides the pattern or code to ribosome for manufacturing the specific proteins.

Mitochondria: These are pleomorphic bodies *i.e.,* their shapes and sizes are highly variable. These are rod, filament or granule-like and size varies from 0.5μ to 10μ. Mitochondria are present in large numbers in the cells except in bacteria and mammalian red blood cells. These bodies are called "the power house of the cells" where aerobic respiration takes place to generate energy by gradual, stepwise oxidation of carbohydrates, fats or proteins. The energy is harvested by the ADP/ATP system and is made available at other sites.

Chloroplasts: In plants chloroplasts are present to carry out the functions for obtaining additional energy from photosynthesis. These contain chlorophyll pigments.

Plastids: These granules are present in plant cells and unicellular animals but not in multicellular animals including insects. Some plastids are coloured due to the presence of red, orange, brown or yellow pigments. These are called *chromoplasts* to distinguish them from colourless plastids, the *leucoplasts*.

Lysosomes: These are spherical bodies of 0.2 μ to 0.8 μ size. Lysosomes contain a number of digestive enzymes which are released when cell is damaged or dies, bringing autolysis of cell.

Centriole: It is present in all animal and plant cells and is visible only during cell division when centrioles duplicate.

Nucleus: This is the most important and prominent part of the cell and is the co-ordinating centre for cellular processes. The major portion of hereditary material is contained in it. Without nucleus the cell cannot function for long and dies. The *nuclear membrane* is double-walled and the space between them is filled with a fluid. The membrane has several pores of 200A° diameter (A°—angstrom *i.e.*, 0.1 millimicron). It is believed that the nuclear membrane is continuous with endoplasmic reticulum. The membrane surrounds the nuclear sap or *nucleoplasm, chromatin network* and *nucleolus*. The chromatin network in dividing cells becomes transformed to *chromosomes*. Nucleolus is a spherical or irregularly-shaped body and contains large quantities of RNA which gives rise to messenger RNA for carrying biological code for protein synthesis.

Some physical phenomena of life processes in cells

Permeability of cell membrane: The living cell maintains a dynamic balance between external environment and chemical and physical activities of its protoplasm. This phenomenon of self regulation by the cell or organism to its surrounding is termed *homeostasis*. Homeostasis can function only if the flow of the external material present in surrounding media like blood, lymph or water is suitably controlled. This control is exercised by the selective permeability of the cell membrane or plasma membrane. Plasma membrane as endoplasmic reticulum is also present around Golgi bodies, mitochondria, lysosomes and other cellular structures. The endoplasmic reticulum makes several compartments within the cell and in each compartment different activities can go on. Proteins may be produced and stored in Golgi bodies., oxidation occurs in mitochondria whereas reduction occurs at the same time outside mitochondria. The two reactions remain separate because of the reticular membrane. The sugar in the cytoplasm cannot be oxidized unless it permeates the plasma membrane around mitochondria.

How do biological membranes regulate exit or entry of materials? It is not always due solely to diffusion *i.e.*, movement due to differential concentration of the material on the two sides of the membrane. At one time it was thought that materials always move from a region of higher concentration to that of the lower concentration. In other words if cell has a lower concentration of a substance say glucose, then glucose from blood which has it in higher concentration, will go to the cell across the cell wall. We now know that cells have various substances at higher concentration than that in blood, and in spite of this materials do not go out of cell but are actively transported into the cell. This *active transport* is a chemical phenomenon and requires an expenditure of energy and is distinct from the *passive transport* which is strictly a physical phenomenon.

Most cells in animal and plant tissues are surrounded by aqueous solution. Normally substances move across the membrane in soluble state; but all dissolved substances are not permitted to pass through the living membrane because of its selective or differential permeability.

The permeability of cell membrane is influenced by other phenomena like surface energy or surface tension, adsorption, colloidal state and viscosity.

Surface energy or surface tension : When two surfaces meet, a tension or energy is created due to molecular action at the interphase. The surfaces may be liquid-liquid, liquid-gas, liquid-solid, gas-gas *etc.* Let us take an example. Water in a beaker meets with air at the surface. The water molecules at the surface are pulled down by water molecules below the surface with greater force producing a more rigid film or layer of water at the surface. It is this phenomenon of surface tension that causes a liquid to take a spherical shape if other antagonistic forces are not involved.

Adsorption : It is also a surface phenomenon by which a substance adheres to the surface of another substance. Enzymes present surfaces to the substrate and chemical reaction takes place on these surfaces. *Absorption* is different. Here the substance is held by cohesion or capillary force. Water is held by a sponge or blotting paper into which the former is absorbed in, but protein molecules are adhered or concentrated on the surface of proteolytic enzyme by adsorption.

Colloids : The term colloid was first used by Thomas Graham in 1861, to distinguish substances like egg albumen from substances that crystallize. According to the present concept colloidal state of a substance is related to the size of its particles and not whether it will crystallize or not. A substance is generally said to be in colloidal state if its particles are 1 to 200 millimicron in diameter. Protoplasm and other body fluids like blood, milk, digestive secretions *etc.*, all form colloidal systems. In living system substances are in solutions or colloidal states. Common salt or glucose dissolved in water is a true solution because it is homogenous throughout and consists of *one phase* only. If mustard or any oil is shaken in water, the oil particles disperse forming a *disperse phase* in water which forms the *dispersing medium*. The oil/water is an example of the two liquid phases which are not stable because after some time the water and oil will separate unless these are held in emulsion state by the addition of an emulsifier which prevents particles of two liquids from uniting. Similarly, stable or unstable dispersion phase can be formed if a solid is dispersed in a liquid medium. In milk, casein forms a stable phase in water. In other words milk is a colloid and not a solution. The stability is imparted because the particle size of dispersed casein is very small. Substances in colloidal state do not pass through semi-permeable membranes by diffusion.

Colloidal state has a great significanse in a biological system. The substance in colloidal state offers large surface area for adsorption of biological catalysts, the enzymes. The surface area of a cube with a 1 cm side is 6 cm^2 but the same mass if broken into particles of colloidal dimensions the total surface area may be nearly 1.5 acres taking one side of the colloidal cube as 1 $m\mu$ or .000001 mm.

CELLULAR RESPIRATION

We have seen that various substances in protoplasm and in its surrounding are in such a physical state that processes in active living cells go on efficiently. The proper physical state of protoplasm is a pre-requisite for its chemical activities. The cell is often termed as the most intricate and efficient factory where highly complex substances are synthesized and broken down by oxidation producing new substances including excretory products. The cell produces controlled energy required by the living system. We will now discuss how energy is evolved from the fuel, how it is harvested and utilized.

Energy production

Respiration is a process by which chemically bound energy of food is released in a metabolically utilizable form of living cell. The respiratory centres at cellular level are mitochondria. The chemical energy of the organic molecules like sugar, starch, protein *etc.*, represents directly or indirectly the stored *solar energy*. During photosynthesis carbon, oxygen and hydrogen from simple substances are combined and the sun provides necessary energy for binding different atoms to form organic molecules. These organic molecules are taken in as food by the animals. At cellular level the C-C bonds of these substances or 'fuel' are gradually broken down to yield the energy. This phenomenon of energy production is somewhat similar to the burning of wood. The heat given out by the burning wood represents the solar energy trapped by the tree when it was living. But a fire is uncontrolled combustion where energy is liberated suddenly, raising the temperatures to high levels, and most of the energy produced in the form of heat and to some extent light, is dissipated into the surrounding.

In respiration, the fuel (carbohydrates, protein *etc.,*) is very gradually broken down with the help of enzymes with the production of very little heat and no light. The temperature remains within the biological limits. Without enzymes, a similar reaction can be obtained in a test tube but the temperature will have to be raised to a level at which life will be destroyed.

Energy from organic bonds

During photosynthesis in plants, complex substances are produced from simple molecules whose atoms are bound together by the energy provided by the sun to form long chain carbon-compounds. During cellular respiration these C-C bonds, one by one are dislocated producing several intermediary products with the release of energy. The removal of energy is brought about by the removal of hydrogen from the organic molecule by a process known as *dehydrogenation*. Hydrogen can be removed only in the presence of a suitable substance generally termed as *hydrogen acceptor*. One such acceptor is oxygen.

Removal of hydrogen (*dehydrogenation*), water (*dehydration*), carbon dioxide (*decarboxylation*) or removal of electrons (a form of ionization) are all oxidative processes. It must be borne in mind that hydrogen acceptors do not start combustion of fuel. The energy evolution in a living cell never stops. It is passed on from parents to zygote. This energy is used for sustaining respiratory processes. A hydrogen acceptor like O_2 of atmosphere will not remove hydrogen from a log of wood because O_2 cannot initiate combustion. Heat by lighting a match must be supplied to the wood for igniting the fuel. This activation energy (heat) causes hydrogen to become loose and combine with atmospheric O_2. The fire creates a self-sustaining chain reaction and the log continues to burn. In the cell, process of hydrogenation is sustained by a system of enzymes. Thus the following events take place during cellular respiration:

(a) Oxidation of cellular material releases hydrogen or electron from the fuel. This hydrogen is attached to one of the several *hydrogen acceptors* or *electron acceptors* like DPN (diphosphopyridine nucleotide), TPN (triphosphopyridine nucleotide), FAD (flavin adenine dinucleotide), FMN (flavin mononucleotide), cytochromes and oxygen. Different fuel fragments donate hydrogen to their own specific acceptors. Substances like pyruvic acid under anaerobic condition act as hydrogen carrier receiving H_2 forming lactic acid or alcohol. This phase can be called as the phase of *hydrogen transfer* (from fuel to 'acceptors').

(b) As a result of dehydrogenation or oxidation, carbon to carbon bonds of molecules slowly break down. Through intermediary steps the multi-carbon fuel is degraded to 1-carbon substance which appears as CO_2. This phase is the *fuel break down phase.*

(c) The oxidation of fuel releases energy, a part of which escapes as heat but rest is harvested or trapped by the creation of high energy bond molecules in the fuel. These bonds are then transferred to ADP/ATP system. The end product of respiration is the creation of high energy-bond phosphate, ATP (adenosine triphosphate) molecule which helps in the utilization of fuel energy in the metabolic activities of the animal. This is the *energy transfer phase* of respiration. The chief excretory products of aerobic respiration are CO_2 and water.

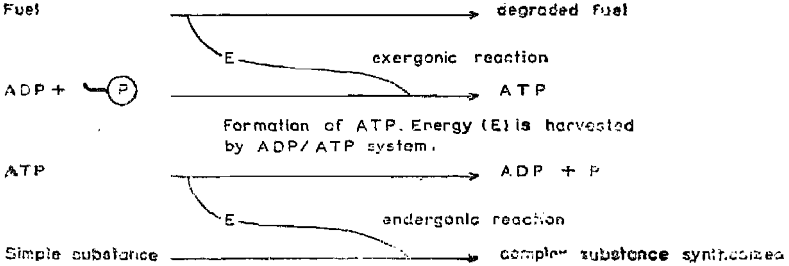
ADP/ATP system

The living organisms contain five major groups of organic molecules *viz.*, adenosine phosphate, polysaccharides, fats, proteins and nucleic acids. ADP (adenosine diphosphate) and ATP (adenosine triphosphate) are derived from a class of substances known as *nucleosides* (a sugar + a nitrogen base) *e.g.*, *adenosine* which is formed by *ribose* (a sugar) and *adenine*. The adenosine can combine with one, two or three phosphate groups derived from inorganic mineral nutrient, phosphoric acid, giving respectively AMP (adenosine monophosphate), ADP and ATP.

AMP and ADP have phosphate groups but the energy required in this phosphorylation is not much. If however, ADP is to change into ATP by the addition of an extra phosphate group a lot more energy is needed from fuel oxidation. When ADP changes into ATP a lot of energy is harvested. This reaction occurs in mitochondria of the cells. The ATP then goes out of the cell and is ready to donate chemical energy for carrying out activities like movement, flight, digestion *etc.* ATP changes into ADP and thus donates a large amount of energy.

Respiration

We have noted that unless phosphate groups are combined with substances, energy evolved during oxidation will not be harvested for metabolic work. Combination of substances with phosphate groups is termed *phosphorylation.* This is an important reaction. Glucose can pass readily through cell wall if it is phosphorylated and changed to glucose phosphate. It is in this form the glucose is metabolically active. The phosphate group in this reaction is provided by ATP which has two low energy (—P) and one high energy (~ P) phosphate bonds as shown below:



Formation of ATP. Energy (E) is harvested by ADP/ATP system.

Formation of ADP from ATP which donates E for metabolism.

ATP = A—A—P ~ P
ADP = A—P—P

When glucose is phosphorylated ATP also donates ~ P (high energy bond), but the glucose gets a low energy bond—P and the remaining energy appears as heat. Such heat losses are unavoidable because ATP alone has to get involved in the phosphorylation of several substances including glucose.

Oxidation of fuel

We have noted that oxidation of fuel brings about gradual breaking of C-C bonds resulting in the release of energy which is harvested by ADP/ATP system so that the energy is in the utilizable form for metabolic processes. We will now briefly discuss the intermediary products of fuel breakdown under anaerobic and aerobic conditions.

This energy-releasing process in the absence of oxygen is called *fermentation* and was a main feature of many primitive organisms at the time when the primitive atmosphere of earth contained little or no free oxygen gas. Fermentation however is found in plants and animals of today including man. General nature of this process was known to ancient man who fermented fruit juices by yeast to produce alcohol and milk to produce cheese.

Yeast under aerobic conditions will multiply and grow at the expense of glucose and produce CO_2 and water but under anaerobic situations it does not grow but continuously produces alcohol and carbon dioxide. Insects are aerobes but can function anaerobically for a limited period. Plant roots survive anaerobically during temporary water-logged conditions.

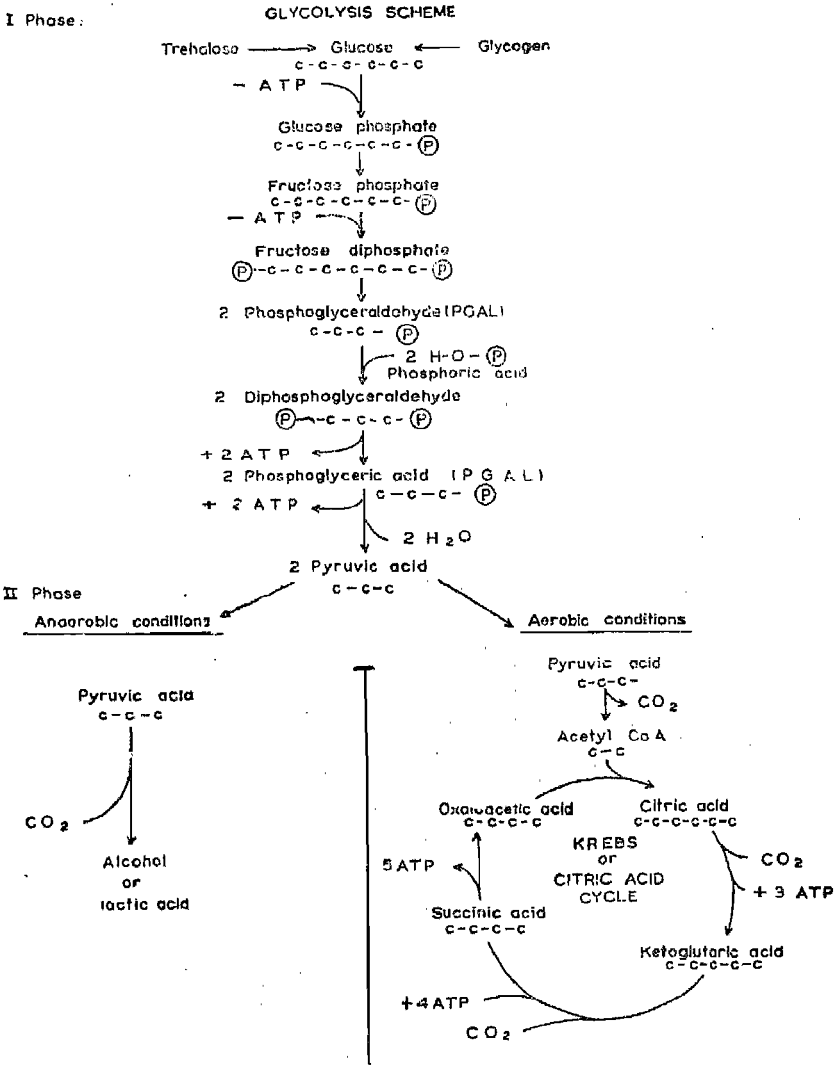
Oxidation of carbohydrates (Glycolysis): This is the chief way of breakdown of carbohydrate fuel. Glycogen or starch or trehalose (in insects) is first converted into glucose. The glucose is phosphorylated to glucose-phosphate which ultimately splits through stages into 3-carbon sugar called *phosphoglyceraldehyde* (PGAL) which is changed to pyruvic acid. The main steps of conversion are shown on the next page.

In the first phase note how 6-carbon compounds are gradually degrading to 3 and then to 2-C. Several steps have been omitted to simplify the discussion. Up to the formation of pyruvic acid the reaction occurs without involvement of oxygen (anaerobic). If reaction proceeds without oxygen in phase II, pyruvic acid changes into either alcohol or lactic acid with the evolution of CO_2. The energy evolved for the formation of ATP is very little and only 2 ATP molecules are gained during the entire reaction.

If phase II has aerobic conditions, pyruvic acid changes into a substance called acetyl co-enzyme A which has 2-C. This combines with a 4-C compound, oxaloacetic acid to form citric acid which successively changes into ketoglutaric acid and succinic acid and then back to oxaloacetic acid which becomes available to receive fresh acetyl Co A. The entire reaction has an involvement of enzymes containing derivatives of B and other vitamins, DPN and TPN. This cyclic reaction is the *citric acid or tricarboxylic cycle*. It is also called Krebs cycle after the name of the scientist, Prof. Hans Krebs of England who received the Nobel Prize in medicine in 1953. Krebs cycle is the main energy yielding reaction. As many as 38 ATP are produced in this reaction for each molecule of glucose burnt aerobically.

It can be concluded that in fermentation CO_2, alcohol and 2 ATP are produced while in respiration (aerobic) CO_2, water and 38 ATP are produced for each molecule of glucose. In cells these reactions take place at a very fast rate. A single molecule of glucose can completely burn within a second. The nature has provided in the biological system, a power house which defies all imaginations of man.

Oxidation of fats and proteins: During respiration besides carbohydrates, other classes of cellular compounds including fats and proteins are burnt. The respiratory processes, like fire have no respect for any class of substance. This destructive process leads to wear and tear of tissues which are



continually replaced or repaired by the formation of body substances by a process called *intermediary metabolism* (anabolism) which is not an energy yielding process.

When fats are used as respiratory fuel, they first change into fatty acids and glycerine. Fatty acid gets converted to acetyl Co A and glycerine (a 3-C substance) then to PGAL.

Thus fats enter the reaction cycle of glycolysis at different stages and contribute in the cellular respiration.

Proteins similarly first break down into their constituent amino acids which undergo a process of *deamination* (removal of amino group-NH_2). The deaminated portion through several reactive processes becomes either pyruvic acid or acetyl Co A and follows the pathway similar to that described in glycolysis.

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12

CIRCULATORY SYSTEM

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There are two types of circulatory systems in the animal kingdom. In many animals the blood travels through special vessels such as arteries, capillaries and veins. This is known as a *closed system*. In insects the blood flows through the body cavity, irrigating the various tissues and organs. This is known as *open system*, and the body cavity through which the blood flows is called a *haemocoele*. There are as a rule no definite arteries and veins with the exception of an aorta-like anterior prolongation of the dorsal vessel which usually divides into terminal branches and the segmented blood vessels of the order Dictyoptera.

Organs of circulation

The diaphragms and sinuses: Generally the haemocoele is imperfectly divided into three sinuses by means of two fibromuscular septa known as diaphragms. One is the dorsal diaphragm which extends across the abdominal cavity above the alimentary canal and the blood-space enclosed by this is known as the *dorsal* or *pericardial sinus* which contains heart. The second is the ventral diaphragm, which stretches across the abdominal cavity just above the ventral nerve cord and the blood-space enclosed by this is known as *ventral* or *peri-*

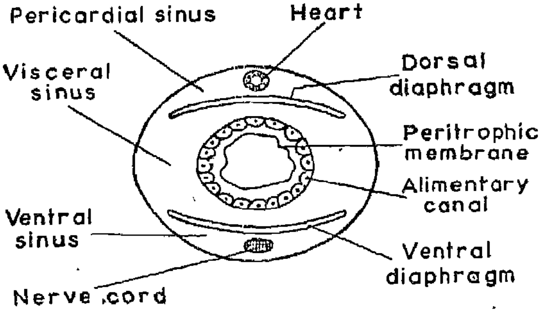

Fig. 12-1. Transverse section of insect abdomen showing sinuses and septa

neural sinus. Between the dorsal and ventral sinuses is the large *visceral sinus* containing the principal internal organs (Fig. 12-1).

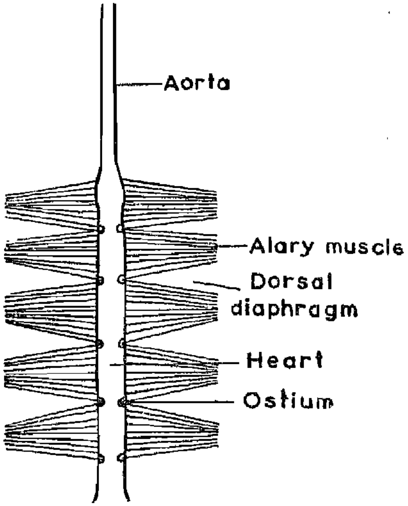

Fig. 12.2. Dorsal vessel showing the chambers of heart and alary muscles.

Pairs of alary muscles, composed of striated fibres and tissues resembling elastic connective fibres, arise from the terga and spread out fanwise over the surface of the dorsal diaphragm (Fig. 12-2). The fibres of one alary muscle meet beneath the heart with those of the corresponding muscle of the opposite side of the body. In some insects these are attached to the walls of the heart. These muscles vary in number, *e.g.*, cockroach 12 pairs, hive bee 4 pairs, *Haematopinus* 3 pairs and larva of *Chironomus* 2 pairs.

The dorsal vessel : It extends from near the caudal extremity of the body, through the thorax, and terminates in the head and is situated along the median dorsal line just beneath the integument and is protected by dorsal diaphragm below. It is a continuous tube, usually closed posteriorly and always open at its cephalic extremity and is divided into two regions *heart* and *aorta.* Heart acts as a pumping organ and aorta as conducting vessel.

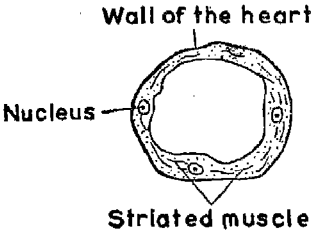

Fig. 12.3. Transverse section of heart showing its histological structure.

Heart—It may be divided into a series of chambers by successive constrictions but in a simple unconstricted tube the segmentation is evidenced by the presence of paired incurrent ostia and the alary muscles. In cockroaches, 12 segments are involved, in aculeate Hymenoptera there are 5 divisions, in Mallophaga, Anoplura and in some Hemiptera, there is only one chamber. The heart is composed of a single layer of cells with large nuclei and striated muscle fibrillae differentiated within the cytoplasm. The cellular layer is bound both externally and internally by a delicate membraneous tunic (Fig. 12-3). The blood enters the heart through lateral inlets or *incurrent osita,* a pair of which is situated at each constriction. The wall of the heart is deffected inwards and forwards at each ostium to form an *auricular valve,* which precludes return flow of the blood into the dorsal sinus. In many insects each pair of auricular valve also functions as *ventricular valve* which prevents the backward flow of the blood in the heart itself (Fig. 12-4). In the larva of *Aeschna* (Odonata) the ventricular valves are separately developed and situated some distance in front of each pair of ostia (Fig. 12-5).

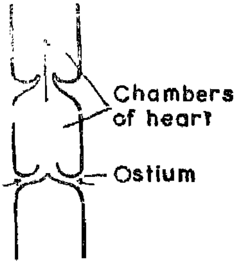

Fig. 12-4. Diagram showing flow of blood in heart chambers.

The Aorta—It is an anterior prolongation of the dorsal vessel and functions as principal artery of the blood. Its junction with the heart is frequently marked by the presence of *aortic valves.* The aorta extends forwards through the thorax to terminate in the heart near the brain. In some insects its anterior extremity is an open funnel like mouth but more usually, it divides into two or more *cephalic arteries,* each of which may subdivide into smaller vessels.

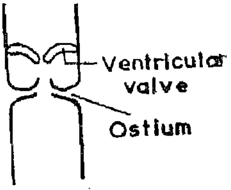

Fig. 12-5. Diagram showing the position of ventricular valves in larva of *Aeschna* (Odonata).

Accessory pulsatory organs: These are sac-like structures situated in various regions of the body and pulsate independently of the heart, ensuring adequate circulation of blood through the appendages. In *Herse* (Sphingidae) and *Dytiscus* (water beetle) these are present just beneath the meso and metathoracic terga. In *Herse,* mesotergal and in water beetle metatergal pulsating organs are well-developed. In Hemiptera these are present in legs. In *Periplaneta* and in other insects including Lepidoptera there is also a pulsatile vesicle at the base of each antenna. In Cyclorrhapha there is a pair of accessory hearts in scutellum and four pulsating organs at the base of the wings.

The Blood

The blood is the only extracellular fluid in the insect body and makes up 15-75 per cent of the volume of the insect. It consists of *plasma* liquid and blood cells or *haemocytes.*

Plasma: Plasma contains 85 per cent of water. It is usually slightly acidic and includes inorganic ions, amino-acids, proteins, fats, sugars, organic acids and other substances in variable amounts. Usually it is transparent but owing to certain pigments, it is commonly tinted with amber, yellow, brown, green, orange and red colours. In some species there is difference of blood colour in the sexes. Green colour is due to chlorophyll dissolved in the plasma and red colour in chironomid larvae is due to haemoglobin. The plasma has got the following functions:—(i) storage of water, which can be used during desiccation; (ii) storage of food; (iii) transportation of food materials and hormones; (iv) mechanical functioning in eversion of protrusible structures *e.g., Ptilinum* in Diptera, penis in male insects and dilation of wings at time of emergence of adult; (v) respiratory role as in chironomid larvae and (vi) *reflex bleeding* in some insects when they are disturbed. Insects have power of emission of blood through articulations of body or pores or slits in the cuticle; it acts as protective function. They usually feign death and the blood which exudes may possess toxic, caustic or other properties which it is believed to render such insects distasteful to their enemies. This function is very much evident in a Coleoptera, certain Hemiptera and Orthoptera and also in aphids where blood is discharged through cornicles.

Haemocytes: Many attempts have been made to classify haemocytes histologically but they are not entirely satisfactory. However, there are four types of common haemocytes (Fig. 12-6).

Proleucocytes—Small, rounded basophil cells, reproduced by mitotic division.

Phagocytes—Large size with hyaline cytoplasm.

Granular leucocytes—Cytoplasm granular, frequently functioning as phagocytes.

Oenocytoides—Large cells with rounded or spherical forms, having a homogenous cytoplasm. They occur in Coleoptera, Lepidoptera, Hemiptera and Diptera but not in the Orthoptera. First three types are generally present in all insects.

Besides these, haemocytes have been divided into different types based upon characters such as general shape (ovoidal, amoeboid, fusiform or vermiform), size, structure of nucleus, staining property of the cytoplasm *etc.* In this way there are 10 recognized main categories and thirty-two types of cells in the larva of *Prodenia* (Noctuidae). In hemipteran bug

(Pyrrhocoridae) these cells are laden with oily droplets or wax and are known as "*Adipoleucocytes*".

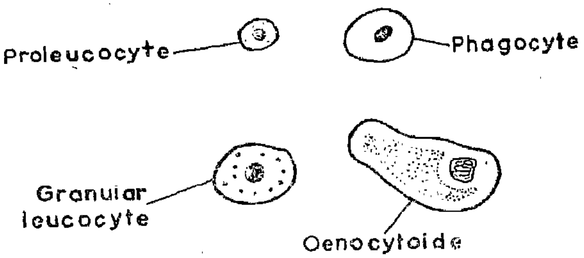

Fig. 12-6. Diagram showing different types of haemocytes

Number of haemocytes present in the blood of various insects varies from 1000 to one million cells per cubic mm.

The principal function of the haemocytes is *phagocytosis i.e.,* ingestion of small solid particles. Besides this, these may help in the defence reaction of insects by devouring foreign particles which are harmful and also fulfil the function of storage by taking fat and glycogen.

Coagulation of blood: The blood being the most vital fluid in an animal, possesses mechanism by which its excessive loss in the event of injury is prevented by a process called coagulation. In higher animals (mammals) this process is initiated by special blood cells called *platelets* or *thrombocytes,* which are very small measuring 2μ-3μ. During injury these platelets come in contact with the foreign surface and disintegrate to release a substance called *thromboplastin* which in turn changes into *thrombin* in combination with *prothrombin* present in blood plasma. The blood contains another soluble protein known as *fibrinogen* with which the thrombin combines to form insoluble protein known as fibrin. The fibrin produces the characteristic blood clot over the injury.

In insects the coagulation is brought about either by the plasma or by the cellular inclusion (haemocytes). In plasmatic coagulation a large number of specialized haemocytes gather at the site of wound to release certain chemicals which bring about coagulation of plasma at the site of the injury. Such a mechanism exists mainly in Orthoptera and Dermaptera. In cellular coagulation the haemocytes do not secrete any substance but instead these throw out pseudopodia at the site of wound. Protoplasmic projections of the adjoining haemocytes join together forming a fine network which is then covered over by a membrane and thus the wound is sealed. In this case, there is no coagulation of plasma. This type is found in Odonata, Coleoptera and Lepidoptera.

Circulation of blood: The heart is the principal pulsatory organ (Fig. 12-7) and undergoes rhythmical contractions which are brought about by the muscle fibrillae of its walls. This rhythm is either myogenic or neurogenic. Contraction of the heart takes the form of a wave of peristalsis which runs forward from the posterior end. It varies from slow to fast. Diastole results from the relaxation of the heart muscle and in some insects it is by the contraction of alary muscles or the elastic tension of their fibres. During diastole the blood enters the heart by the incurrent ostia which may exclude some or all of haemocytes. On contraction the expanded lips of these ostia act as valves so that the blood is prevented from returning through these to the pericardial sinus and is propelled forward. In its forward passage, some blood leaves the heart by the excurrent ostia and lateral segmental vessels when these are present; the remainder enters the cephalic haemocoele from which a part is pumped into the

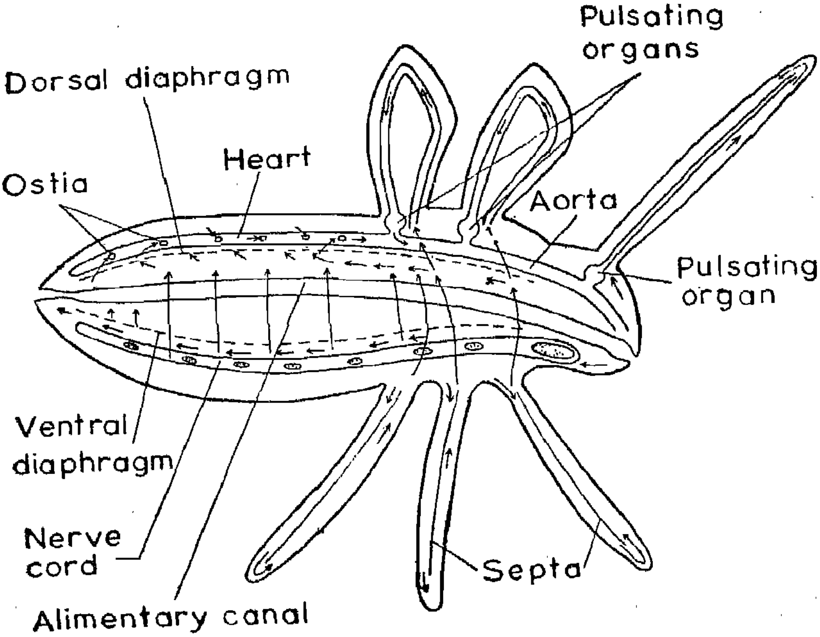

Fig. 12-7. Insect with fully developed circulatory system showing circulation accomplished by heart and accessory structures.

antennae by accessory pulsatile organs; the circulation of blood through the wings of many insects occurs in definite channels between the inner walls of the veins and the enclosed trachea and takes the form of a distally directed flow in the front of half of the wing and a return flow along the posterior margin. It is brought by the thoracic pulsatile organs which aspirate blood from the posterior part of the wing base. Circulation through the legs may be achieved through accessory hearts at their bases, helped by movements of the legs and the presence of diaphragms within. In these cases blood enters on one side, flows the length of the appendage and empties on the other side. Blood movements into the appendages are aided also by the respiratary movements, so that the "pulse" in the legs may synchronize with respiratory contractions and not with the heart beats. From the head and thorax the blood flows backwards in the haemocoele and is probably assisted in this direction by undulatory movements of the ventral diaphragm.

Control of heart beat

As already stated the dorsal vessel or heart is the main organ associated with the circulatory system through which a part of haemolymph is rhythmically propelled from backward to forward. The direction of blood flow in heart and the frequency of its beat are subjected to modification depending upon various internal and external factors. The flow of blood at times follows a reverse direction (forward to backward) especially when carbon dioxide accumulates in the anterior region. Similarly, the rate of heart beat also fluctuates. In several insects the wall of the heart does not possess nerve supply suggesting that the rhythmic contraction of heart is probably a myogenic phenomenon. In some species, however, the heart receives nerve supply from segmental ganglia. In such cases, the contraction could be both myogenic or neurogenic. It has been observed that in almost all cases an isolated heart from which all nerve supply has been cut off is capable of beat without losing the normal rhythm. It is likely that like mammals, insects probably also possess a pace-maker which gives out periodic stimulus to the heart. How does the heart beat in isolation is still a mystery and cannot be explained even on basis of the presence of pace-maker. Some workers have indicated that a rhythmic control of heart is probably a hormonal phenomenon.

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13

EXCRETORY SYSTEM

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Many waste products of metabolism are of no value to the organism or would be harmful if allowed to accumulate. The process of elimination of these waste products is *excretion* and the mechanical devices developed in the organism for this process constitute *excretory system*.

The eliminated substances of insects are solid, liquid or gaseous in form. The principal gas expelled as a waste is *carbon dioxide, and the principal liquid is water. Solid excreta* are given off mostly in crystalline masses or in aqueous solutions. These include nitrogenous and non-nitrogenous salts. The organs that serve to eliminate these substances are known as excretory organs and these are: (i) Malpighian tubules, (ii) integument, (iii) tracheal system and (iv) wall of the alimentary canal. In addition, there are accessory organs such as nephrocytes, fat bodies, oenocytes and cephalic glands.

Principal organs of excretion

1. **Malpighian tubules:** The Malpighian tubules were first discovered by an Italian anatomist Malpighi and were named after him. These are almost universally present among insects except Collembola, some Thysanura and Aphids. These (Fig. 13-1) are long, slender and blind tubules lying in the haemocoele where these are freely bathed by the blood. These open at their proximal extremities into the intestine, near the junction of hindgut and midgut (Fig. 13-1a). Distally these are usually free but in some insects the distal ends of the tubules are closely applied to the hindgut (Fig. 13-1b). This type of condition is known as *cryptonephridial* condition and it occurs in the larvae and adults of many Coleoptera, in some Hymenoptera larvae, Neuroptera and almost all Lepidoptera larvae. Cryptonephridial system probably enables the insects to conserve water by withdrawing moisture from faeces. Each Malpighian tubule in some insects (reduviid bugs) is differentiated into two or three parts (Fig. 13-1c). Very frequently there is a vesicular enlargement or ampulla at the bases of each tubule (Fig. 13-1d).

Number, size and type of Malpighian tubules vary from insect to insect. The primitive number is believed to be six, but fewer or more may be present. Some Odonata and

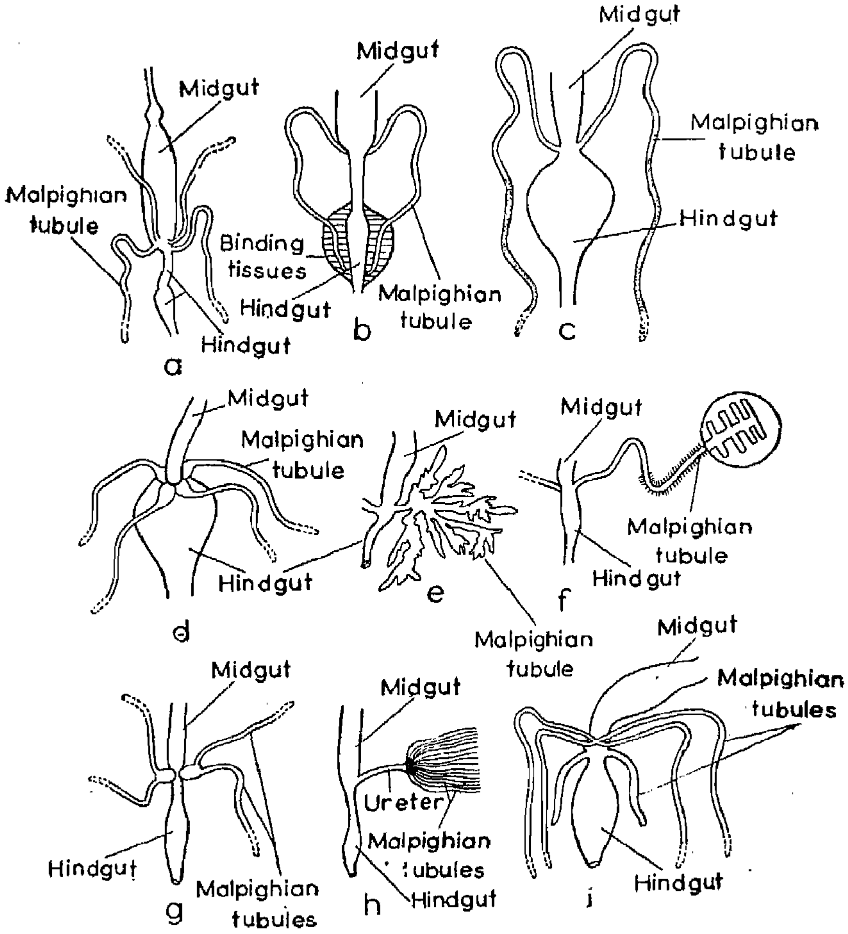

Fig. 13-1. Different types of Malpighian tubules.

Orthoptera may have as many as two hundred tubules. When a few tubules are present, these are generally long, sometimes longer than the body. When a great many are present these are often short. Regardless of number and length, their total surface area is of the order of four hundred to five hundred square millimetres per milligram of insect weight.

The typical number of Malpighian tubules present in various orders is as follows:

Anoplura, Thysanoptera, Hemiptera, Diptera 4, Psocoptera, Coleoptera 4-6, Isoptera 2-8, Thysanura 4-16, Lepidoptera 6, Dermaptera 8-20, Odonata 50-70, Orthoptera 30-120, Hymenoptera 6-20 in ants and over 100 in Aculeata (Hymenoptera). There are certain exceptions such as Coccoidea, with two and Culicidae with five Malpighian tubules. In Protura, Diplura and Strepsiptera, these are represented by papillae.

Malpighian tubules are often modified in different ways. Sometimes these are arborescent as in *Galleria* (Lepidoptera) (Fig. 13-1e) or give off short closely packed diverticula as in *Melolontha* (Coleoptera) (Fig. 13-1f). Very frequently the tubules unite in groups of two or three and these may open into a common ampulla or bladder, which discharges into the intestine as in *Dysdercus* (Hemiptera) (Fig. 13-1g). Sometimes all the tubules converge to open into a common ureter as in *Acheta* (Orthoptera) (Fig. 13-1h). In *Haltica* and *Donacia* (Coleoptera) four of the tubules discharge into a common ampulla while the remaining two shorter vessels have isolated insertions (Fig. 13-1i).

Histologically these are generally regarded as derivatives of the ectodermal proctodaeum but according to some these are similar to mesenteron. Still some others believe them to originate from an undifferentiated zone of cells between midgut and hindgut.

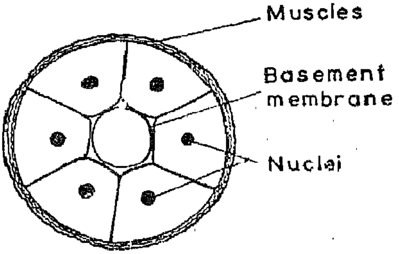

Fig. 13-2. Transverse section of a Malpighian tubule.

When viewed in transverse section a Malpighian tubule is seen to be composed of about three to eight large and variably-shaped epithelial cells with prominent nuclei (Fig. 13-2). The latter increase in size during development and may become palmate or form giant endopolyploid nuclei. Where each cell borders the lumen of the tubule, it possesses a brush or honeycomb border, the appearance of which may vary over different parts of the tubule. The epithelial cells also show an outer striated zone in many insects and rest externally on a basement membrane (*tunica propria*) which is covered by a peritoneal coat often containing muscle fibres. The muscles which are sometimes difficult to detect, may run in bands or as a reticulum over the whole tube or are restricted to the proximal region; these appear not to be innervated but are responsible for peristaltic movements of the tubules. In Thysanura, Dermaptera and Thysanoptera there are no muscles and peristalsis does not occur.

In insects, where each Malpighian tubule is differentiated into two regions *viz.*, one-third proximal and two-third distal, there is also distinct differentiation histologically in these parts. The cells of proximal region bear a brush border while those of distal part have honeycomb border. It is only in the lower region one finds uric acid granules. The cells of ampulla, wherever present, have outer wall prolonged into processes that project down into the lumen of proctodaeum. The outer surfaces of the tubules are richly supplied with fine branches of tracheae.

The primary function of the Malpighian tubules is excretion and regulation of the internal environment by controlling the ion and water balance in the blood and by removing nitrogenous wastes. The requirements and details of these processes vary with the food and water intake and with the external environment. Insects living in water or in damp humus have little need for water conservation, but those exposed to sunlight and air are subject to desiccation and must conserve water. These tubules come in direct contact with blood and withdraw water, dissolved salts, amino acids and nitrogenous wastes. These materials pass down the lumen of the tubules into the hindgut. Some or all of the water, salts and nutritive substances are reabsorbed in the proximal part of the tubules or in the hindgut while nitrogenous wastes pass out with faeces. Cryptonephridial tubules probably serve to conserve water by facilitating its removal from faecal material in the hindgut. Waste excreted by these, include large quantities of uric acid, small amounts of urea and ammonia, more rarely allantoine and other materials as mineral salts and water which are not needed to maintain suitable balance in the blood.

Secondary functions of the Malpighian tubules are known in some insects. Modified cells in the tubules of some larval Chrysomelidae (Coleoptera) produce a sticky secretion that aids ambulation, while some females of the same family enclose the eggs in a similar secretion. The spittle surrounding immature Cercopidae (Hemiptera) is also a Malpighian tubule secretion. Many immature Neuroptera and late larvae of several species of Coleoptera produce silk from specialized cells in the Malpighian epithelium. In the luminous larvae of *Bolitophila* (Diptera) the light is emitted by thoracic organs formed by free ends of Malpighian tubules lying just beneath the transparent cuticle of thoracic region.

2. **Integument:** Moulting in insects is suggested to be a process of excretion, particularly of nitrogenous waste products. Chitin constitutes probably thirty to forty per cent of the insect cuticula. Even substances like pigments are the final products of metabolism transferred to certain parts of the integument and deposited instead of being excreted. It

is quite clear that with each moult insect loses a large amount of nitrogen and also other substances that are deposited in exuviae. In spite of many mechanical devices involved to facilitate respiration, part of respiration always takes place through some part of the ectoderm and in this way ectoderm acts as excretory tissue for the elimination of carbon dioxide and water.

3. **Tracheal system:** It is concerned with uptake and use of oxygen by the tissues and liberation and disposal of the carbon dioxide. So, technically the process of elimination of carbon dioxide and some water is also excretion. Respiratory devices developed in insects in true sense also act as excretory organs.

4. **Alimentary canal:** It has been observed that there is an accumulation of crystalline bodies in the walls of midgut of various insects. These bodies are salts of calcium and uric acid. In either case the bodies are probably excretory products or excess substances that cannot be utilized. Insects make little use of calcium absorbed from food. It is probable that these substances are eliminated directly through the walls of hindgut, but the most of the intestinal excretion takes place through the Malpighian tubules.

In Collembola and aphids Malpighian tubules are absent and the gut plays an important role. Epithelial cells of midgut are also casted off with each moulting and these cells contain excretory materials.

In Strepsiptera although Malpighian tubules are present yet excretory process is accomplished by large binucleate cells scattered regularly in dorsal walls of the posterior midgut.

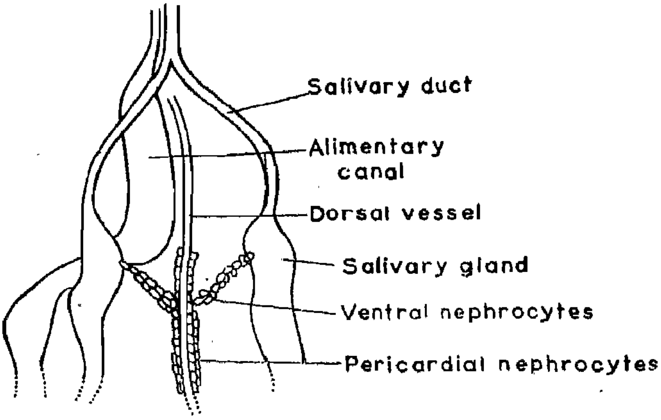

Fig. 13-3. Dissection of insect showing the position of nephrocytes.

Accessory organs of excretion

1. **Nephrocytes:** These are groups of cells found in localized regions of insect body (Fig. 13-3). These occur in two principal groups: (i) the dorsal or pericardial and (ii) the ventral nephrocytes (Fig. 13-3). The dorsal nephrocytes are arranged in a linear series one on either side of the heart in the pericardial sinus; such cells are present in the immature stages and adults of most insects. The ventral nephrocytes occur in the form of a chain of cells (Fig. 13-3, 4) suspended in the body cavity below the intestine and attached by its two extremities to the salivary glands. These occur in dipterous larvae.

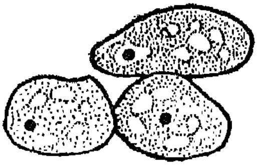

Fig. 13-4. Magnified structure of ventral nephrocytes.

2. **Fat bodies:** These are composed of irregular masses or lobes of rounded or polyhedral cells (trophocytes) which are usually vacuolated and contain inclusions of various kinds. In many insects the fat body is built up of tightly compact cells., in others it is a more or less laminate tissue with numerous lacunae, or it may take the form of loose stands. In colour it may be white, yellow, orange or greenish. In many insects (Fig. 13-5) it is possible to distinguish an outer or *parietal layer*, beneath the body wall, and an inner or *visceral layer* which surrounds and enters between the various organs. In some larvae the parietal layer

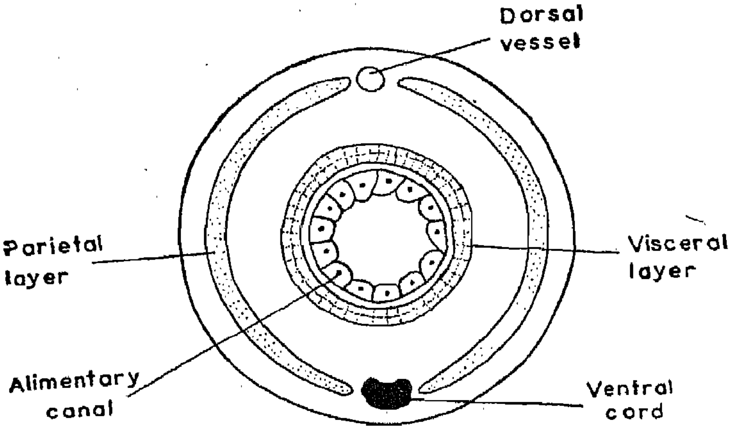

Fig. 13-5 Transverse section of an insect showing the layers of fat bodies.

is interrupted at each segment but visceral layer forms a continuous sheet.

In some insects deposits of uric acid or urates appear in the fat body. In Collembola, *Blatta* and hymenopterous larvae, these excretory granules are formed in specialized *urate cells* of fat body as the Malpighian tubules are either absent or not functional. In other cases, even though the tubules may be functional, urates may also be deposited in the *trophocytes*. In the larvae of Apocrita, Lepidoptera and cyclorrhaphan Diptera, the urates of the fat body are transferred to the Malpighian tubules at pupation. Calcium salts also accumulate in the fat body of phytophagous dipterous larvae. The fat body carries out the functions of storage excretion.

3. **Oenocytes:** These are large cells found in the body cavity of most insects either arranged in groups or scattered individually among the tissues of the abdomen where these are usually associated with the fat cells. Some earlier workers reagrded these as excretory organs. The latest thinking is that, their function is involved in secreting the cuticulin layer of the epicuticle and in forming the grease layer in cockroaches.

4. **Labial glands:** These are tubular structures situated in the head of Collembola opening by a common duct above the base of labium (Fig. 13-6). These consist of an upper region of "saccule" with flat-tened epithelium, communicating with a long coiled tube or by labyrinth", the cells of which resemble those of Malpighian tubules.

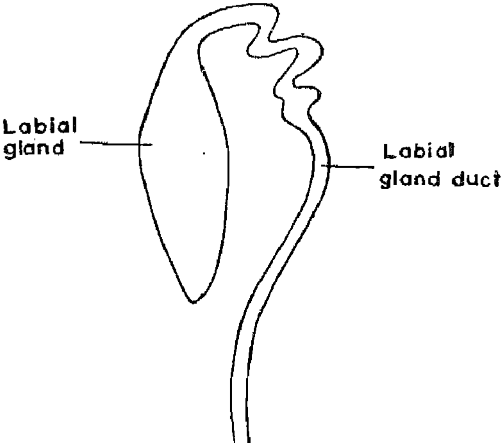

Fig. 13.6. Structure of a labial gland.

Physiology of excretion

The main function of excretion is to maintain internal environment in such a condition that all life processes are carried out without any hindrance. When nutrients or water become excess these too are removed as excretory products. The excretion controls various constituents of the cells and the body fluids by eliminating excess of metabolites which may or may not be harmful, and also by reabsorbing useful materials which invariably accompany the excretory products at the time of its elimination from the body. The elimination of CO_2 is also an excretory phenomenon but is dealt separately under respiration which is carried out by an entirely different set of system. As a rule excretion deals with the elimination of nitrogenous waste products, excess of salts and water, pigment, and even essential amino acids if these are present in excess quantity in the body. This regulatory function of excretion operates at cellular level and the selective permeability of cell wall plays an important role by permitting the entry or exist of substances formed in the cytoplasm. In almost all insects, with a few exceptions like aphids the Malpighian tubules absorb excretory metabolites and release them into the lumen of the organ. In terrestrial forms the cells of Malpighian tubules and those of rectum are modified to reabsorb water and essential ions before the excretory products are thrown out of the body. The situation is quite different in aquatic insects and in fluid feeders where removal of excessive water is one of the main functions connected with osmoregulation. The significance of osmoregulatory processes in insects and the role of hormones is discussed in greater detail elsewhere in the book (Chapter 14).

Wigglesworth has described in detail the anatomy and physiology of Malpighian tubules in *Rhodnius prolixus* and described how nitrogenous metabolites from the body tissue reach the Malpighian tubules and are eliminated without the loss of water and essential ions. In this insect two-third of distal portion of Malpighian tubules absorbs excretory material from the haemolymph and this is transported through the cytoplasm of the cells and secreted into the lumen in liquid form. In one-third proximal portion uric acid is precipitated in the granular form which finally reaches the hindgut.

The uric acid (principal excretory product) in the blood chemically combines with sodium and potassium carbonates to form sodium and potassium urates which being soluble in water are readily absorbed by the distal part of Malpighian tubules. The urates in turn are secreted into the lumen. By the time the fluid reaches the proximal part of the Malpighian tubules the uric acid is precipitated by the action of carbonic anhydrase in the alkaline pH prevailing in the proximal region where water and carbonates are reabsorbed so that these can be utilized again in the next excretory cycle. The semi-solid uric acid particles are pushed into the hindgut by the action of a large number of cilia present in the lumen of Malpighian tubules. The cilia also help in retaining the excretory fluid in this region long enough to permit precipitation of uric acid and reabsorption of water salts or any other essential materials which may have accompanied excretory products during their transmission from the body fluid to the lumen of Malpighian tubules.

Aquatic insects do not face the danger of water losses but they in turn have to evolve mechanism for removing excess of water and other soluble substances which tend to upset the internal environment of insect body. The urine in these forms therefore invariably is fluid. The loss of K and Na ions does occur but these are absorbed from the surrounding water partly by the activity of rectal lining or by the structures like gills or papillae. The insects which live in salty medium tend to lose water from the body because of concentration being higher in the surrounding media. This loss is replenished by utilizing the free water of the food and also by the nature of intestinal epithelial cells which absorb water against concentration gradients.

Main products of excretion are nitrogenous substances like ammonia, urea, uric acid rarely amino acids and allantoine. Of these, uric acid is the most important excretory product in terrestrial insects while ammonia is excreted by aquatic forms where water conservation is no problem. In blowfly larvae the large amount of ammonia is excreted directly into the gut without the intervention of Malpighian tubules.

Ammonia: Ammonia is produced by the breakdown of urea into ammonia and CO_2 by enzymatic action. In *Tinea* and *Lucilia* it constitutes about 10 per cent of the urine. This is a poisonous substance, its elimination therefore requires a large quantity of water.

Urea: It is the main constituent of vertebrate urine but is present in very small quantity in insects. Most insects do not contain the enzyme, allantoicase which breaks allantoine into urea during the metabolism of arginine.

Uric acid: Uric acid is insoluble in water but its sodium and potassium salts are readily soluble. It is excreted from the body in the form of sodium or potassium urates. Uric acid is the main constituent of urine of terrestrial insects, reptiles and birds which are often referred to as *urotelic* animals. Urotelic mode of excretion is of great significance during pupal stage when the metabolic rate is very high, but excretory products cannot be removed. As it is practically insoluble in water, it can be easily stored in specialized tissues of the body without interfering with body fluid. Further, chemically it contains least amount of hydrogen per atom of nitrogen and therefore requires less water, which donates the hydrogen part of uric acid molecule. Uric acid excretion therefore can be regarded as a special physiological adaptation for water conservation in terrestrial insects.

Allantoine: In blowfly larvae and *Dysdercus* the haemolymph is quite rich in uric acid but urine chiefly consists of allantoine. In these insects the uric acid is broken down by an enzyme called uricase into allantoine which is soluble in water and can therefore be readily excreted out. Allantoine is a major constituent of urine of fish and amphibians.

Honey dew: Several homopteran insects give out honey dew as an excretory product. These insects possess highly modified alimentary canal to cope with large amount of cell sap which they have to imbibe during feeding. Certain portions of the gut are freely permeable to water and other soluble constituents like amino acids and sugars. These are normally absorbed in the first part of the alimentary canal and are readily excreted out either through Malpighian tubules or through abdominal tubercles. In membracids the presence of filter chamber cuts short the passage of these substances from the foregut directly to the hindgut.

At one time it was thought that the honey dew contains nutritionally useless metabolites but the results of analyses of honey dew produced by different insects, clearly establish that it contains substantial amount of amino compounds, fructose, glucose and even glucose-1-phosphate.

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14

HORMONAL REGULATION OF ELECTROLYTES AND WATER IN ANIMALS

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ALL animals, including insects and man possess the capacity to retain electrolytes like sodium, potassium, calcium chlorides *etc.*, in their bodies. They are able to maintain requisite quantity of these ions, with little alterations, for relatively long periods even when the diet either contains high contents of sodium chloride and other salts or suffers from their deficiency and thus the electrolytes are kept in balance. Under abnormal situations, as in pathological condition electrolyte balance is disturbed and may cause even death.

Water like other body constituents, is in a dynamic state both in extra and intracellular regions. However its quantity in a particular location remains within a critical range. The electrolytes play an important part in the movement of water across the cell membrane and this indicates the presence of a common or closely-related mechanism controlling electrolytes and water within the living system. Further these mechanisms are influenced by several hormones.

Hormones have a marked influence on such basic life-processes as growth, development, reproduction, energy utilization and cell-permeability. We now shall deal with the role of hormones in cell-permeability concerning transport of electrolytes (inorganic ions) and water across cells. Some of the hormones, known to have regulatory effect on electrolytes and water present in the cells, tissues and extracellular fluids of vertebrates are listed below:

1. Vasopressin or antidiuretic hormone (ADH) from neurohypophysis.
2. Aldosterone from adrenal cortex.
3. Thyroid hormone from thyroid.
4. Parathormone from parathyroid.
5. Oxytocin from neurohypophysis.
6. Deoxycorticosterone (DOC) from adrenal cortex.
7. Insulin from Islets of Langerhans in pancreas.
8. Adenocorticotrophic hormone (ACTH) from adenophysis.
9. Somatotrophic hormone (STH) from adenohypophysis.
10. Androgen and estrogen from gonads.

Of these ten, only the first four *viz.*, ADH, aldosterone, thyroid hormone and parathormone are of major importance in this connection as the other six have only limited role in the regulation of electrolytes and water. Two examples given below will illustrate the point:

Antidiuretic hormone: The mode of action of this hormone in producing antidiuresis varies in different species of vertebrates; it acts on skin, kidney, tubules and urinary bladder in Amphibia; reduces filtration through renal glomeruli in crocodiles; causes both reduced glomerular filtration and increased tubular resorption in birds; in mammals it mainly increases the tubular resorption. This hormone is of physiological utility in the regulation of water balance in all the vertebrates except fishes.

In mammals, in which extensive studies have been made, ADH reduces the volume of urine and increases its concentration by encouraging water resorption through the epithelial cells of the distal portion of renal tubules. Insufficiency of ADH produces diabetes insipidus, a disease in which profound disturbance in water metabolism occurs, such diabetics consume large quantities of water (polydipsia) and eliminate large quantities of dilute urine. The symptoms can be corrected by the administration of ADH and this confirms the role of ADH.

Aldosterone and deoxycorticosterone: These hormones from adrenal cortex are known as mineralocorticoid hormones in view of their significant role in mineral metabolism. These tend to increase the urinary volume (diuretic action). Aldosterone acts both on renal and extrarenal (body fluid, blood and intestine) sites. Insufficiency of this hormone causes excess loss of sodium and chloride through the renal tubules but there is less clearance of potassium and water; this results in increase of Na^+ and Cl^- in urine and their reduction in blood and tissues, while K^+ and water increase in blood and tissues, specially skeletal muscles and decrease in urine. At the extrarenal sites, water and K^+ move into tissue cells and become immobilized while blood becomes more concentrated owing to the loss of fluid blood pressure falls and renal glomerular filtration rate diminishes.

Adrenalectomized animals have reduced ability to tolerate an extra water load. The urinary elimination is delayed causing toxic syndrome. This probably results in part from the accumulation of water within the tissue cells and in part from increased renal reabsorption (antidiuresis).

Aldosterone influences Na^+, K^+, Cl^- and water transport not only through urinary but also through other tissues both *in vivo and in vitro*. It also alters the Na^+ and K^+ ratio in sweat and salivary gland secretions.

Intake of NaCl with drinking water partially corrects the symptoms by (i) replacing the Na^+ and Cl^- lost through urine and (ii) by producing diuresis that tends to flush out the accumulated K^+.

Water regulatory mechanism in insects has been studied in several groups. Both *in vivo* and *in vitro* investigations clearly indicate that insects, like the vertebrates, possess a regulatory system to control the movement of water across the cells mainly of body wall, Malpighian tubules and rectum. As a result of investigations during the last decade it has been possible to demonstrate that the regulatory mechanism resides in a factor or factors of neuroendocrine origin.

It is now an accepted fact that water and several electrolytes enter the Malpighian tubules from the haemolymph into the rectum where water and ions are reabsorbed and returned to the body fluid.

In several insects, it has also been demonstrated that portions of Malpighian tubules are specialized in someway and filtration occurs in one portion while reabsorption occurs in another portion to a certain extent *e.g.*, in *Rhodnius, Corcyra* sp.

Maddrell (1963) gave the first detail description of a water-balance hormone in insects. He demonstrated that in *Rhodnius prolixus,* the hormone is produced by neurosecretory cells in the posterior region of mesothoracic ganglionic mass. Swelling of the midgut following a blood meal causes distention of the abdomen; this provides stimulus for the release of a diuretic hormone in sufficient concentration to bring about maximum diuresis within a few minutes. The hormone is destroyed or excreted out by Malpighian tubules and diuresis stops as soon as the distention of the abdomen is reduced. Similarly hormones from thoracic ganglia increase urine production by the Malpighian tubules in *Corethra* (Diptera), (Gersch, 1967).

The rate of water absorption by rectum is higher in hydrated and lower in dehydrated cockroach. Dehydration of cockroaches by lowering the relative humidity of environment, by supplying with hypertonic saline, or by withholding water from food gave similar results suggesting an antidiuretic response (Wall, 1967; Wall and Oschman, 1967). Brain extracts from dehydrated cockroaches produced an increase in rectal water absorption showing an antidiuretic property. The extracts of corpora allata, metathoracic ganglia and last abdominal ganglia from hydrated insects also produced an increase in the rate of water absorption but similar extracts from dehydrated cockroaches had less activity.

Recent studies by Mordue (1969) and others on *Schistocerca gregaria* have clearly demonstrated the presence of diuretic factors in the extracts of corpora cardiaca. It has dual action: on the one hand increasing the tubular excretion and on the other reducing rectal reabsorption.

The blood volume of a normal locust depends upon the water content of the food. Starved insects show little neurosecretory activity and have very low level of excretion. Feeding the starved locusts activates neurosecretion resulting in an increased rate of excretion.

Mordue (1969) believes that in desert locust any reduction in diuresis is brought about not by antidiuretic factors but by the cessation in the release of diuretic factors or breakdown of these factors already present in haemolymph. Some workers have however reported antidiuretic hormones in locusts. Delphin (1963, 1965) suggested the production of antidiuretic factor/s in the neurosecretory cells of ventral nerve cord of *Schistocerca*. Cazal and Girardie (1968) found that extracts of both the cerebral neurosecretory cells and corpora cardiaca increase rectal reabsorption in *Locusta migratoria*.

The few examples cited above, although contradictory at places, clearly indicate that neuroendocrine organs in insects are sites of the production of one or both diuretic and antidiuretic hormones. These hormones are produced at different sites as mentioned below :

(i) Antidiuretic hormones are produced in the brain and other ganglia of ventral nerve cord in *Iphita* (Nayar, 1960), *Schistocerca* (Delphin, 1963, 1965), *Locusta* (Cazal and Girardie, 1968) and *Periplaneta* (Wall, 1967).

(ii) Diuretic hormones have been shown to be secreted from the brain and thoracic ganglia in beetle, *Anisotarsus* (Nunez, 1956), *Rhodnius* (Maddrell, 1963), *Schistocerca* (Highnam *et al.*, 1965; Mordue, 1969) and *Dysdercus* (Berridge, 1966).

(iii) Both diuretic and antidiuretic hormones are believed to be produced from the brain and abdominal ganglia in certain other insects (Raabe, 1959; Mills, 1967).

(iv) Antidiuretic activity was found in the extract of corpora allata of cockroach (Wall, 1967). The hormone decreases the rate of secretion in Malpighian tubules and increases rectal resorption. Similar findings by Unger (1965) and Vietinghoff (1967) clearly show that D component of neurohormone in *Carausius* has a diuretic effect on its Malpighian tubule and antidiuretic effect on the rectum. In both these insects the hormones accelerate excretion through Malpighian tubules and reduce rectal resorption. In vertebrates specially birds the action is however different than that found in insects. In birds the ADH reduces while in *Schistocerca* it increases urine volume. The mode of the dual action of the hormone is not clearly understood. Since elaborate chemical isolation techniques have made it possible to separate a number of active principles from crude extracts, it is not unlikely that further sophistication may enable future workers to isolate from the so called single substance, more than one active component, one affecting a particular site (glomerulus of bird and Malpighian tubule of insect) and the other a different site (renal tubule of bird and rectum of insect).

On the basis of our present knowledge of the function of Malpighian tubules and rectum, neurosecretory regulation of excretion can be successful only if the neurosecretory factors act upon both organs. On the analogy of similar processes in certain vertebrates like birds, it is possible that a single substance acts on both these structures to produce a diuretic or an antidiuretic effect. In the former, diuretic factor would increase the rate of water entry into the lumen of Malpighian tubule and at the same time reduce rectal resorption. The reverse process would bring about antidiuretic effect. Perhaps this could be extended to epidermal cells, since a lower permeability of these cells would reduce evaporative water loss. In *Rhodnius*, the permeability of the epidermal cells to water is envisaged to be under neuroendocrine control (Maddrell, 1965, 1966).

The dual action of neuroendocrine factors, antagonistic to each other has also been reported in birds among vertebrates. The ADH reduces glomerular filtration and increases rectal resorption, a process similar to the dual action reported by Mordue (1969) in *Schistocerca gregaria.* Unger (1965) found two components C and D in the neurohormone; C has antidiuretic and D diuretic effect on the Malpighian tubule.

There is hardly any information about the hormonal regulation of electrolytes in insects. Since it is an accepted fact that in most of the animals regulation of water is dependent upon the regulation of electrolytes (regulation of electrolytes and water is linked in vertebrates) it would not be unreasonable to postulate that a similar mechanism should be operative in insects as well. Insects would not be an exception to this in general mechanism.

In the present state of our knowledge, it is not possible to indicate the precise mode of action of the hormones in relation to electrolyte and water regulation in insects. One thing however, has emerged with certainty that in insects too, like the vertebrates, hormones play a significant role in regulating water and consequently electrolytes. The mechanism has yet to be elucidated by intensive and extensive research in this field.

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15

REPRODUCTIVE SYSTEM

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The reproductive system in insects contributes little or nothing towards the welfare of insects which possess them. In insects the main function of reproductive system is to provide succeeding generations. In higher animals like mammals, reproductive organs produce gonadotropic hormones which influence metabolism, the development and maintenance of secondary sexual characters, menstrual cycle, pregnancy and development of milk glands. In insects no such functions are carried out by reproductive organs which are only involved in the production of germ cells (ova and sperms), formation of yolk in females and spermatophores in males. The system also provides mechanism for the transfer of sperms to and storage in the body of female.

As a rule male and female reproductive systems are structurally distinct in adults. During embryonic and early post-embryonic stages these organs appear quite similar in both the sexes. In Thysanura, male and female reproductive systems look very similar even in the adult stage. In spite of morphological variation some of the organs of reproductive systems of male and female are homologous as given below:

	Male	*Female*
1.	Paired testes composed of follicles (sperm tubes)	Paired ovaries composed of ovarioles (egg tubes)
2.	Paired vasa differentia	Paired oviducts
3.	Median ejaculatory duct	Median oviduct
4.	Accessory glands	Accessory glands and Colleterial glands
5.	—	Spermatheca (sperm pouch)
6.	—	Bursa copulatrix (copulatory pouch)

Some insects, however are hermaphrodite where both male and female organs occur in the same individual. In *Perla marginata* (Plecoptera) hermaphrodite condition exists in young males in which female organs degenerate at maturity and only male organs persist. *Icerya purchasi* (Homoptera) is an example of permanent functional-hermophrodite condition. The females are capable of self fertilization by the sperms produced in testes of the same individual. In some other insects like coccids, aphids, thrips *etc.*, young ones are produced parthenogenetically from unfertilized eggs.

FEMALE REPRODUCTIVE SYSTEM

The female reproductive system consists of ovaries, lateral oviducts, median oviduct, spermatheca, bursa copulatrix and accessory glands (Fig. 15-1).

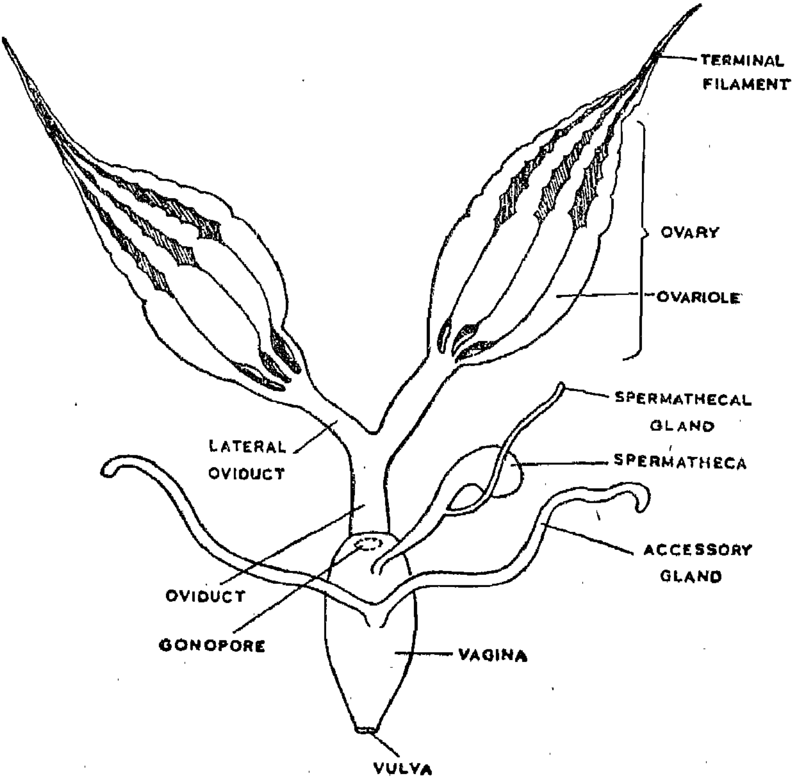

Fig. 15-1. Typical female reproductive system of an insect.

Ovaries. Generally there are two ovaries, each consisting of varying number of ovarioles. Aphids contain only one ovariole, tsetse fly 2 and some species of termites more than 2000. Ovarioles are absent in Protura and Collembola where

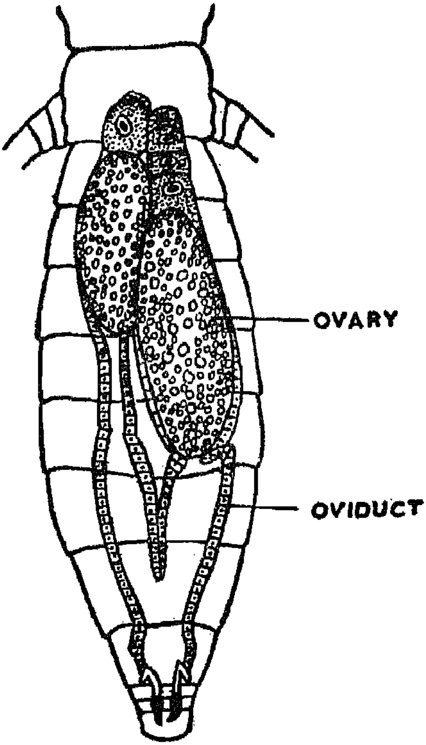

Fig. 15-2. Reproductive system in Protura.

ovaries are represented by a pair of sacs (Fig. 15-2). Each ovariole (Fig. 15-3) is enveloped by double-layered cellular wall. The outer wall called *Ovarial sheath* has an abundant supply of tracheae. The inner layer called *tunica propria* is elastic in nature. Each ovariole has at its terminal end a filament which unites with other filaments to form a *suspensory ligament*. The ligament is attached to the body wall or to the dorsal diaphragm and thus helps the ovaries to remain suspended at the proper place.

An ovariole or egg tube (15-3) consists of the following parts:

Germarium : This is the anterior most region below the terminal filament containing primordial or undifferentiated cells. These give rise to three types of cells: 1. germ cells developing into oogonia and finally into oocytes or egg cells; 2. nutritive nurse cells or trophocytes and 3. follicle cells or cystocytes.

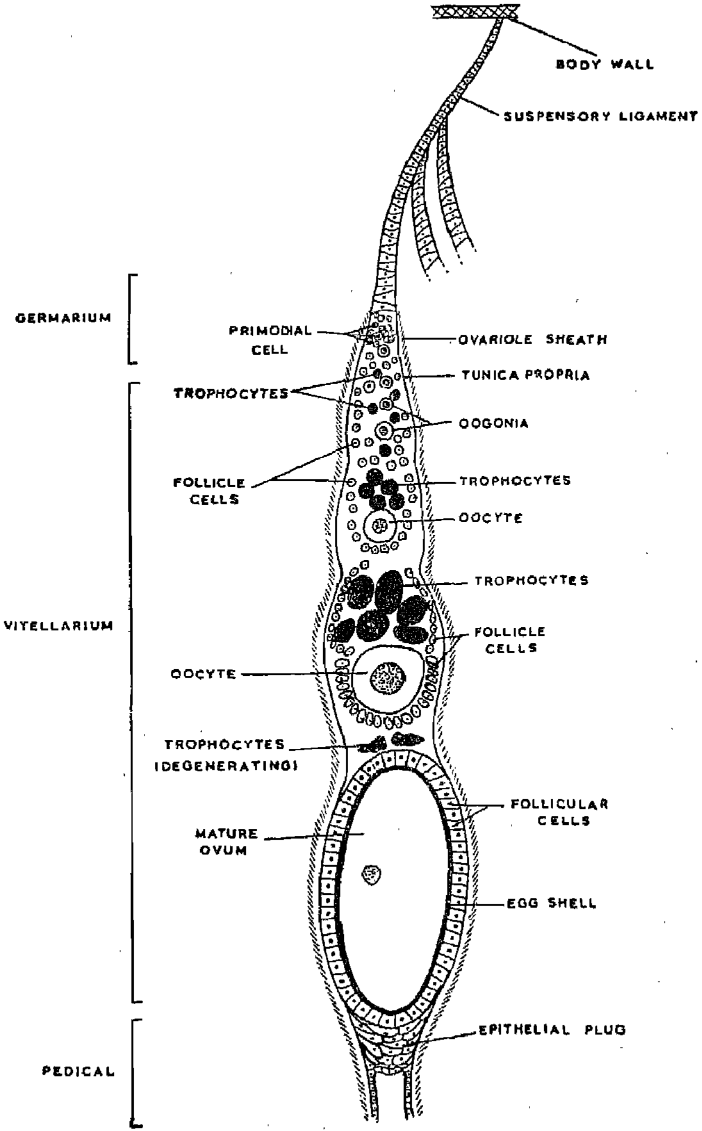

Fig. 15.3 Histology of an ovariole.

Vitellarium: It occupies the major part of the ovariole and contain large number of oocytes (which finally form ova or eggs) in different stages of their development. In the anterior region of vitellarium the nurse cells and oocytes remain mixed up and assume an axial position but the follicle cells take the peripheral position. In the posterior region oocytes are enclosed by the follicle cells to form a definite sac or follicle layer. The nurse cells absorb the nutrients from the haemolymph through the follicular cells and transit them to oocytes. The follicular cells secrete the material which forms the egg shell. In some cases they provide nutrients to the oocytes, specially in insects where nurse cells are wanting.

Pedicel : , The pedicel or stalk of the ovariole is short duct communicating the egg tube with the lateral oviduct. It is mesodermal in origin and develops a lumen connecting with that of a duct but closed at the upper end beneath the epithelial plug of the egg tube. At the time the first egg is ready to be laid the cells of both the plug and the returning wall of the pedicel are dissolved to give a passage to egg from egg tube to oviduct.

There are three types of ovarioles found in insects. The classification is based upon the presence or absence of nutritive cells and on the location of these cells when present.

1. *Panoistic*. In this type of ovarioles nutritive cells are absent (Fig. 15-4a). The oocytes are nourished by the secretion of the follicular cells. This is regarded as a primitive condition and is found in Diplura, Orthoptera, Isoptera, Odonata and Siphonaptera.
2. *Polytrophic*. Here nutritive cells are present and alternate with oocytes (Fig. 15-4b). This is found in Neuroptera, Coleoptera, Hymenoptera, Diptera and Lepidoptera.
3. *Acrotrophic or Telotrophic*. The nutritive cells are present but are crowded at the apex of each ovariole (Fig. 15-4c). Sometimes protoplasmic connections extend from the nutritive cells to the developing oocytes; through these connections nourishment is transmitted.

Oogenesis. The primordial cells in the ovariole pass through different stages of development before becoming mature ova. This developmental phenomenon is called oogenesis (Fig. 15-5). The primordial cell divides to give rise one oocyte, trophocytes (nutritive nurse cells) and follicular cells. The oocyte enlarges at the expense of the secretion provided by the nutritive cells, and in due course of time becomes surrounded by follicular cells.

The oocyte proceeds backward in the ovariole where yolk is deposited and it undergoes further growth and enlarges in size. The nucleus of oocyte grows into enormous size and is often called as *germinal vesicle*. In older oocytes the germinal

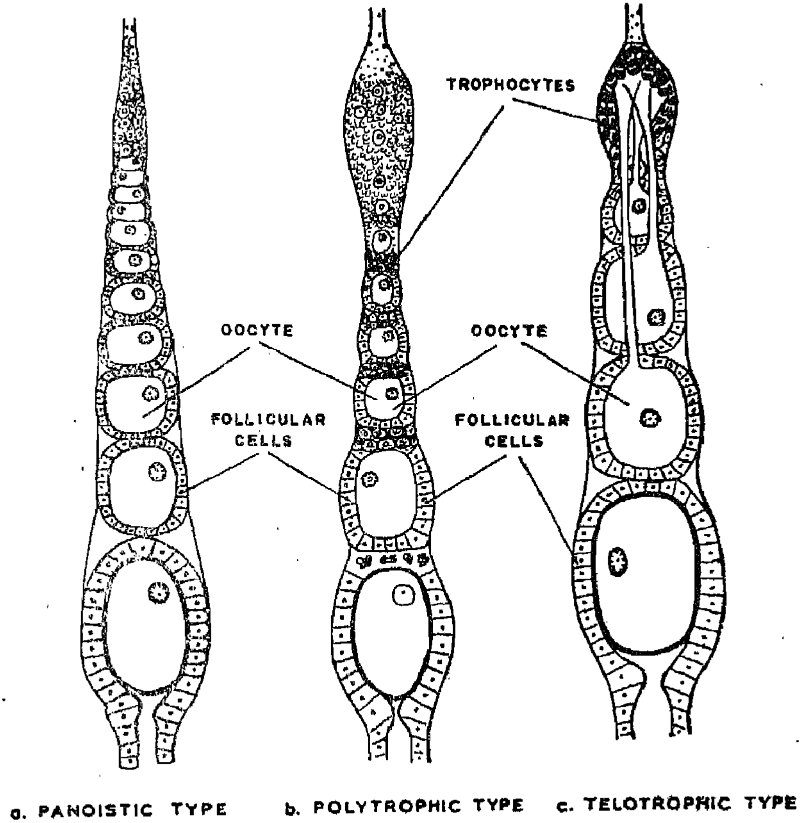

Fig. 15-4. Different types of ovarioles.

vesicle becomes relatively small. In most insects, the maturation division is not complete in oocyte while in the ovariole, this division takes place after it leaves the ovariole. In some insects like *Cimex* (bedbug) the maturation division is completed in the ovary itself probably because of haemocoelic fertilization.

Deposition of egg shell material: When the egg formation is nearing completion, follicular cells secrete egg-shell material which is deposited on the egg. The shell consists of two layers, *viz.*, endochorion and exochorion which are adhered together by means of the cement layer containing tanned protein. The outermost material of endochorion consists of resistance lipoprotein and often shows hexagonal imprints of follicular cells.

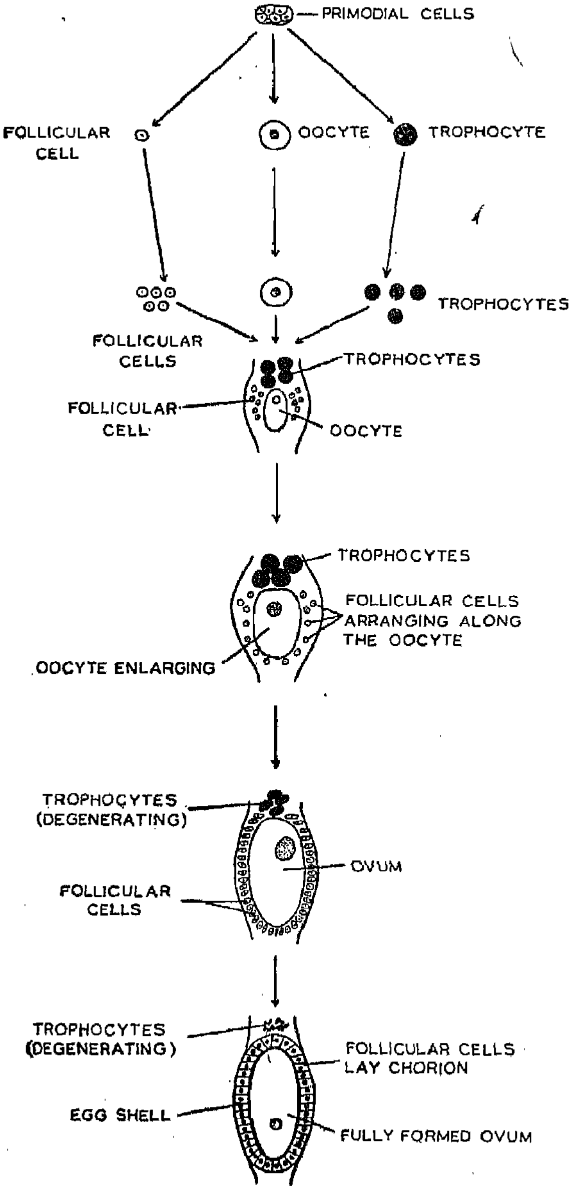

Fig. 15-5. Generalized scheme of oogenesis in an insect.

Reabsorption of oocytes : In several species of insects belonging to orders Orthoptera, Diptera, Hymenoptera, Coleoptera the developing oocytes disintegrate and are reabsorbed by follicular cells of ovariole under adverse conditions like starvation. In parasitic Hymenoptera reabsorption takes place if female is unable to deposit the eggs on a suitable host. This phenomenon is an example of adaptation to the nutritive economy. The more specific the parasite is towards the host, the more prevalent is the reabsorption process.

Lateral Oviducts: Proximal ends of the ovarioles of each ovary join to form a lateral oviduct on each side. The wall of oviduct is glandular and muscular. In grasshoppers this structure becomes elongate for storing fully formed eggs.

Median Oviduct: Two lateral oviducts combine to form a median oviduct which in Dermaptera opens directly outside on VIIth segment. This is a primitive condition and the opening is called *gonopore*. In most of the insects, however, the median oviduct does not open directly to outside but instead into a tubular *genital chamber* or *vagina* formed by an invagination of body wall from VIIIth segment. The vagina opens outside and the opening is termed *vulva* which serves both the purposes of receiving the sperms and discharging the eggs and the true gonopore lies internally and is marked by the insertion of spermatheca. However, in some Lepidoptera there are two openings *viz., vulva* to receive the sperms and *oviporus* for the discharge of eggs from the oviduct.

Bursa copulatrix : In Some insects the genital chamber or vagina develops a separate pouch called *bursa copulatrix* into which sperms are received during copulation. Thus these insects have two reproductive openings in female, one vulva for receiving the sperms and other oviporus for discharging the eggs. This condition is prevalent in ditrysian Lepidoptera (Fig. 15-6) and water beetles.

Spermatheca: Since in insects eggs are not inseminated at the time of mating but receive the sperms at varying length of time afterwards, female is provided with a receptacle where sperms are stored and maintained in a viable condition. These sperms are liberated during the entire ovipositional period of the female. This organ is called *Spermatheca* or *receptaculam seminalis* which is an invagination of body wall of the eighth abdominal segment. The shape, size and number of this organ vary in different insects. The usual number is one but two are present in *Phlebotomus* and *Dacus* (Diptera) and three in *Culex* (Diptera). It is often associated with certain glands or its epithelium is glandular in nature. The secretion of the gland or the epithelium provides nutrient to the sperms. The spermatheca opens into genital chamber through spermatic duct which is muscular and forms a special pumping mechanism

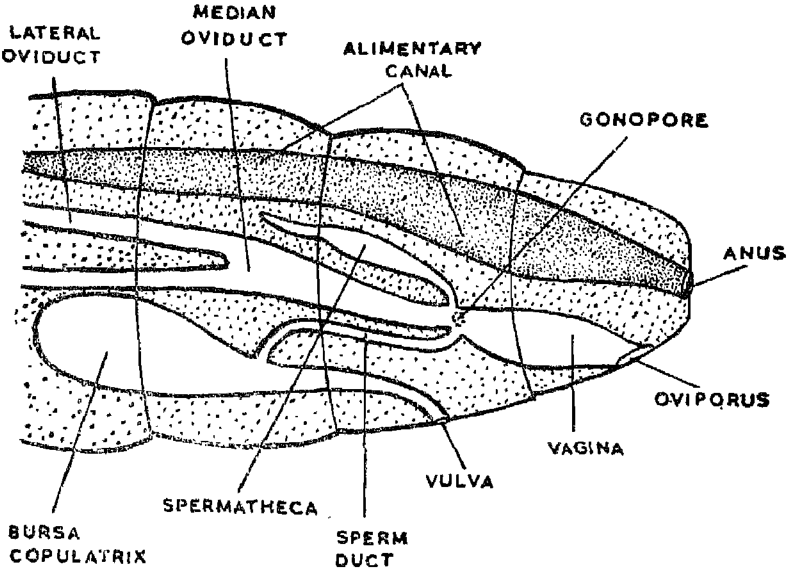

Fig. 15-6. Reproductive system in ditrysian Lepidoptera.

for liberating small quantities of sperms as the eggs are deposited by the female.

Accessory glands: Besides the spermathecal glands mentioned above one or two pairs of additional glands are associated with the reproductive system. These glands are *colleterial glands* which in majority of insects produce sticky material for providing attachment of eggs to the substrate on which they are laid. In cockroaches and mantids they secrete material for the formation of the ootheca. In *Chironomus* they secrete a gelatinous substance for enveloping egg masses. The frothy material which covers the egg mass of the desert locust is also produced by these glands. In rare cases, as in honey bee, ant and several other hymenopterous insects these glands produce poison for paralysing the prey or for defensive purposes. The trailing pheromones in ants are also produced by the accessory glands. These glands are modified as "milk gland" in *Glossina palpalis* where a single larva is retained in the enlarged genital chamber (uterus). The milk glands provide secretion for the developing larva (Fig. 15-7).

MODIFICATIONS OF FEMALE REPRODUCTIVE SYSTEM

Although the reproductive system of all insects have similar basic plan yet modifications of different types are found in different orders. These modifications commensurate with the

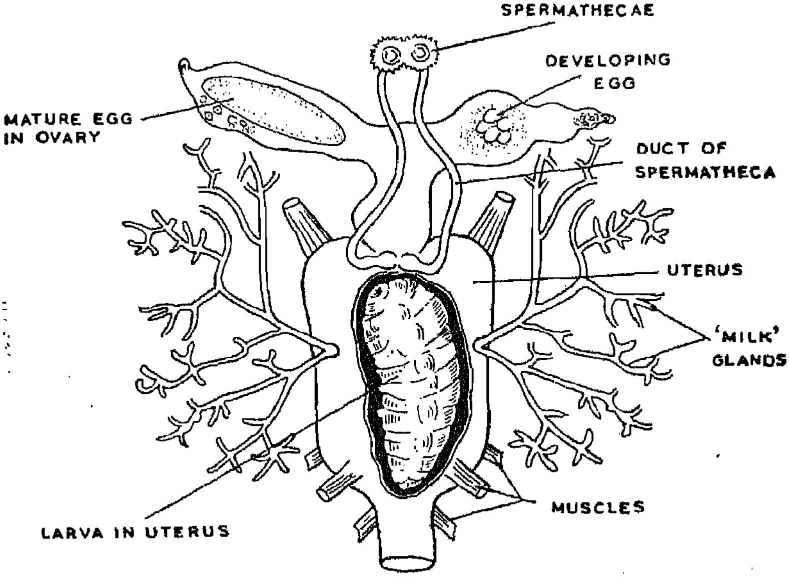

Fig. 15-7. Female reproductive system in *Glossina*.

function and habit of the insects. The simplest form is found in Diplura (Japygidae) where ovarioles are metamerically arranged (Fig. 15-8), and the two oviducts join just before opening at the eighth segment; accessory glands are absent. In Protura there is one pair of panoistic ovaries, lateral oviducts and a short median oviduct, which opens outside between 11th abdominal segment and the telson. However, Collembola presents unique situation where ovaries are not composed of ovarioles but are sac-like with a lateral germarium which produces oocytes. According to some authorities the ovaries are not probably homologous with those of other insects.

In higher orders, ovaries contain 4 to 50 ovarioles. In bedbug, the female reproductive system is very much modified. It possesses a special pouch known as *mesospermalege* or *organ of Ribaga* or *Berlese* in ventral part of the abdomen. This structure does not have any connection with reproductive system at all, but during copulation sperms are deposited in it. The sperms penetrate the wall of the organ and reach the haemococle from where they enter the spermatheca through its wall (Fig. 15-9).

In Diptera female organs undergo various levels of modifications. Number of ovarioles are numerous in oviparous flies while very few in larviparous forms. In *Glossina* the genital

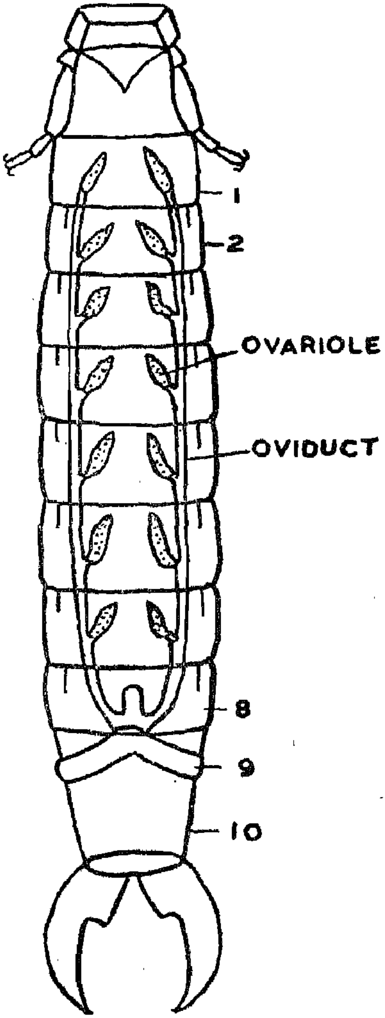

Fig. 15-8. Reproductive system in Diplura.

chamber is modified to form a large uterus like structure in which a single egg develops into fully mature larva which pupates as soon as laid (Fig. 15-7).

MALE REPRODUCTIVE SYSTEM

The male reproductive system consists of a paired testes

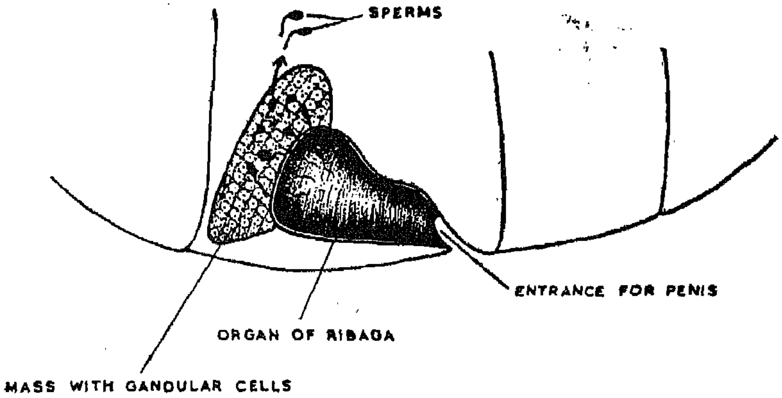

Fig. 15-9. Organs of haemocoelic insemination in female *Cimex*.

with sperm tubes, vasa deferentia and seminal vesicle, median ejaculatory duct and accessory glands (Fig. 15-10a).

Testes: In Apterygota size of the testes is practically the same as that of ovaries but in Pterygota testes are very much smaller. They lie in the abdomen; dorsal, ventral or lateral to the gut and are held in position by attachment of tracheae

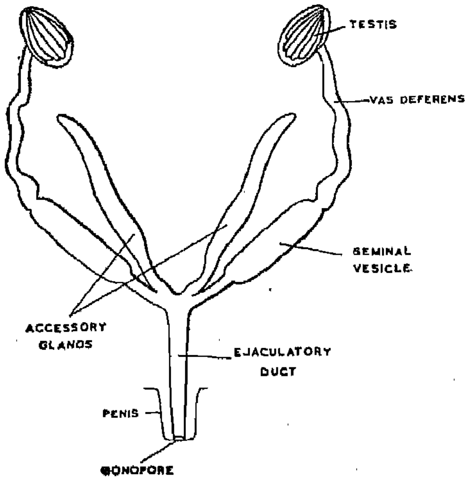

Fig. 15-10a. Typical male reproductive system of an insect.

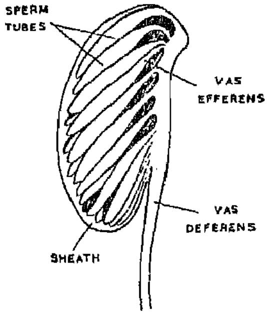

Fig. 15-10b. Structure of a testis.

or fat bodies. Suspensory ligaments found in ovaries are absent in testes. The two testes are separate but in certain Lepidoptera, Orthoptera and Hymenoptera they are invested in a membrane to give an appearance of a single mass. The testis consists of a number of sperm tubes or follicles which join to vasa deferentia by vas efferens (Fig. 15-10b). Sperms are produced, developed and stored in these tubes whose epithelium absorbs nutrients from the haemolymph for the developing sperms.

The sperm tube can be recognized histologically into the following four zones according to the stage of development of sperms (Fig. 15-11a, b).

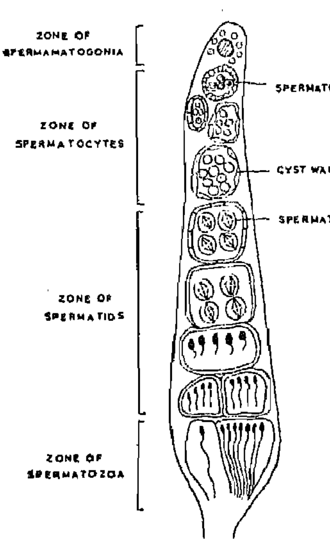

Fig. 15-11a. Testicular Follicle showing different zones.

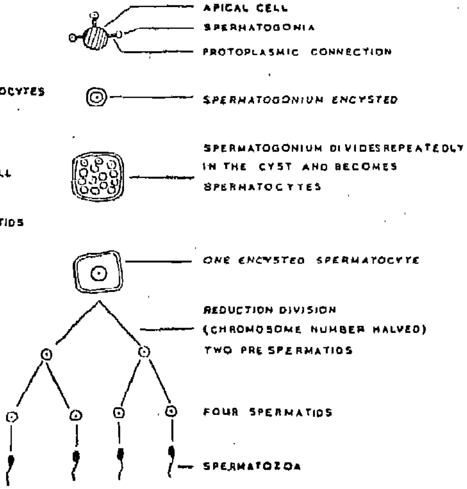
APICAL CELL
SPERMATOGONIA
PROTOPLASMIC CONNECTION
SPERMATOGONIUM ENCYSTED
SPERMATOGONIUM DIVIDES REPEATEDLY IN THE CYST AND BECOMES SPERMATOCYTES
ONE ENCYSTED SPERMATOCYTE
REDUCTION DIVISION (CHROMOSOME NUMBER HALVED)
TWO PRE SPERMATIDS
FOUR SPERMATIDS
SPERMATOZOA

Fig. 15-11b. A schematic representation showing process of spermatogensis in insects.

1. *Zone of spermatogonia or germarium :* This is at the distal end of the sperm tube or follicle and contains germ cells which divide to produce primary spermatogonia. In some insects like Orthoptera, Hemiptera and Lepidoptera large apical cell is present in the apex of germarium. This cell provides nutrients to the developing spermatogonia and thus serve as a nurse cell or specialized trophocyte.

2. *Zone of spermatocytes* : The spermatogonia move backward to occupy this zone. Here each spermatogonium gets enveloped into a cyst and repeatedly divides to give rise to a number of *spermatocytes.*

3. *Zone of maturation* : In this zone spermatocytes undergo reduction division giving rise to prespermatids which further divide and become spermatids. One spermatocyte produces four spermatids. All these processes occur inside the cyst of the spermatocytes.

4. *Zone of transformation* : This is the zone characterized by the transformation of spermatids into spermatozoa. The latter can be distinguished by the presence of tail or flagellum. In the early stage, the spermatozoa remain inside the cyst but later they break through the cyst wall and become free and lie in the proximal extremity of sperm tube. They still remain in a bundle and their heads are covered by gelatinous material. The lashing movement of the tail becomes very fast when sperms reach vas efferens where they perhaps get more oxygen. It is likely that certain enzymes act on the gelatinous material over the heads and set spermatoza free shortly before the act of mating.

Vasa deferentia and seminal Vesicle

The follicles of each testis open independently through short vas efferens into a common tube called vas deferens. The two vasa deferentia join together to form a median ejaculatory duct. The epithelium of vasa deferentia is externally enveloped by circular muscles. In some insects like grasshoppers the posterior part of these ducts enlarges to form seminal vesicle for the storage of the sperms. In some Diptera and Dermaptera, there is a single common seminal vesicle formed by the union of the two ducts. In Coleoptera, vas deferens is a long coiled tube and is often referred as epididymis for storing sperms.

Median ejaculatory duct

This is formed by the invagination of the body wall and is connected to a common duct into which the two vasa deferentia open.

Accessory glands

Associated with male reproductive organs are one to two pairs of accessory glands. In some insects, like cockroaches and mantids these glands form densely branched structure referred as mushroom shaped glands. The secretions of accessory glands are discharged as liquid in which sperms are mixed. This facilitates sperm transmission from male to female. In several species the secretion hardens and forms spermatophores.

These glands are wanting in Apterygota and some dipterans including *Musca*.

Transfer of sperm to female

In majority of insects males inseminate the females by directly depositing sperms mixed with liquid secretion. All Orthoptera, several species of Heteroptera, Trichoptera and Lepidoptera, however have retained a primitive method of insemination in which sperms are contained in a special capsule known as *spermatophore*. These are formed by accessory gland's secretion. In Collembola, male deposits the spermatophore on the ground quite independently of the female. These are picked up later on by a passing female and introduced into its reproductive organs. In Grylloidea and Trichoptera spermatophores are deposited in the genital canal of female during copulation. The sperms escape the capsule and enter the spermatheca. In species of louse (*Linognathus*) it is deposited directly in the spermatheca. In *Locusta* spermatophore is produced by the male during mating and the female accessory glands also help in its formation. In Odonata the mode of transmission of sperms is greatly modified to suit copulation during flight. The true genital opening of the male reproductive organs is situated on the 9th segment. Before copulation, male dragonfly flexes the abdomen ventrally to bring the genital opening in direct contact with the third abdominal sternum which contains a sclerotised tubular structure known as functional penis (accessory copulatory genitalia). The sperms are introduced in the chamber of this organ from where they are transmitted to the female, which during flight places its gonopore in direct contact with accessory genitalia.

The third method of sperm transfer is the haemocoelic insemination in which sperms are deposited directly into haemocoele or to a special internal pouch from where the sperms escape into haemocoele. In *Cimex* such an organ is called Organ of Berlese or Ribaga or mesospermalege which is situated in 4th abdominal segment and has a slit like opening on the posterior edge of sternum. In *Alloeorhynchus* the penis enters the vagina and punctures its wall to discharge the sperms into haemocoele. In all cases of haemocoelic insemination the sperms are disposed in the haemocoele and ultimately reach the ovariole and fertilize the eggs either by penetrating the follicular epithelium or by congregating at the genital canal of female. In the latter case the eggs are fertilized during their passage through common oviduct.

SPECIAL MODES OF REPRODUCTION

In most of the insects eggs are fertilized within the body of the female and deposited outside for subsequent development. This generalized rule however, is not applicable in certain specialized cases which are being dealt below:

Viviparous reproduction

This is a condition where insects instead of laying eggs, produce either larvae or nymphs. This mode of reproduction occurs due to the fact that the eggs are retained in oviduct or uterus (modified vagina) till they hatch. In some cases as in *Glossina* (Diptera) even the entire larval development may take place within the female. The vagina here is enlarged to form a uterus containing milk glands for providing secretion for the development of the larva (Fig. 15-7). In Strepsiptera (stylops) and some species of parthenogenetic Cecidomyidae (*Miaster*), eggs develop in the haemococle of the female. Viviparity is represented in various forms in the class Insecta and is a regular feature among Aphididae, Diptera, Strepsiptera and parasitic Hymenoptera. It can be grouped into four main categories described below:

(a) *Ovoviviparity* : This is a condition where the eggs are retained in the genital tracts so that when they are laid, the embyros have reached the advanced stage of development with the result that the larvae hatch immediately after oviposition. This condition is found in *Lecanium*, *Cimex* (Hemiptera), *Sarcophaga, Hylemya, Termitoxenia* (Diptera) and several other insects chiefly belonging to order Diptera. Sometimes, due to abnormal conditions, eggs may be held back even in truly oviparous Diptera, so the eggs may hatch inside and the larvae are deposited. Such a condition should not be considered as a normal phenomenon of ovoviviparity. In *Termitoxenia* a single large egg is produced with sufficient yolk to provide nourishment for the larva to enable it to moult up to third instar within the egg. The egg hatches into fully developed larva immediately after oviposition and pupates in a few minutes. Ovoviviparity, which forms a link between oviparity and viviparity differs from normal oviparous condition only in the retention of eggs inside the genital tracts.

(b) *Adenotrophic viviparity* : This type is found only in *Glossina* and pupiparous Diptera where eggs are retained in the enlarged vagina (uterus). The embryo develops inside egg and larva on hatching is retained in the uterus where it is nourished by secretion of accessory glands modified into milk glands. The larva when fully developed is laid outside for pupation (Fig. 15-7).

(c) *Pseudoplacental viviparity* : This is a condition where eggs, being devoid of chorion and yolk, are provided with special structures called pseudoplacentae formed by the embryonic or maternal tissues. In the initial stages the egg is nourished inside the ovariole by a single nurse cell. As the embryo advances in age, the follicular chamber of the ovariole also enlarges. At this stage pseudoplacentae are formed which remain in close contact with the developing embryo. The larva does not feed orally but derive nourishment through these structures. The fertilization of the egg takes place in the ovariole itself except in parthenogenetic forms. This type of viviparity is found in Aphididae, *Hemimerus* (Dermaptera), *Hesperoctenes* (Heteroptera) and *Archipsocus* (Psocoptera).

(d) *Haemocoelous viviparity* : This type of viviparity is characterised by a special feature that the development of embryo occurs in the haemocoele of the mother. It is prevalent throughout the order Strepsiptera and larval cecidomyids *e.g., Miaster, Oligarces* (Diptera) which reproduce paedogenetically. In all these insects oviducts are absent and ovaries lie freely among the fat bodies. Mature oocytes escape into the haemocoele by the rupture of ovarian walls. The eggs are devoid of chorion and yolk, but are enveloped in trophic membrane through which nourishment passes from the female haemolymph. The females of Strepsiptera are highly modified and adapted to endoparasitic life. These are larviform, legless and enclosed in the cuticle of last larval instar. Its head remains protruded out of the body surface of the host. Male is winged and inserts the aedeagus through the host and deposits the sperms in female haemocoele where mature eggs are fertilized. The larvae hatch within the body of female and escape into a brood pouch formed by the ventral surface of female and its last instar cuticle. They ultimately emerge through an opening of brood canal (Fig. 15-12). The developing larvae never attack any maternal organs. In larval *Miaster* and *Oligarces* the eggs develop in haemocoele but larvae feed on maternal organs and finally escape cutting through the integument (Fig. 15-13).

Paedogenesis

In some insect species, larvae and pupae are capable of producing either the eggs or larval forms parthenogenetially. This mode of reproduction by the immature forms before attaining the adult stage is called *paedogenesis*. However in some insects, females never become winged, as in coccids, lampyrids and Strepsiptera and resemble the larval forms. The reproduction in these individuals is often referred to as *pseudopaedogenesis* as this does not fall under the category of true paedogenesis because the females are fully mature adults even though externally have larval characters and are often fertilized.

In larval Cecidomyidae and pupal Chironomidae the developing ovarioles produce large number of larvae which bore through the walls of the ovaries into the haemocoele of the parent. The larvae develop at the expense of maternal tissues and they escape out breaking through the integument.

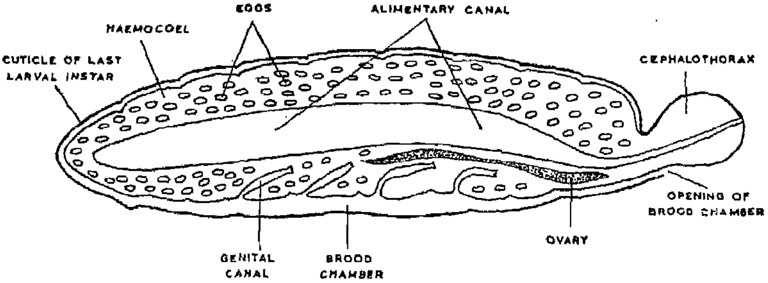

Fig. 15-12. A female strepsipteran showing polyembryony.

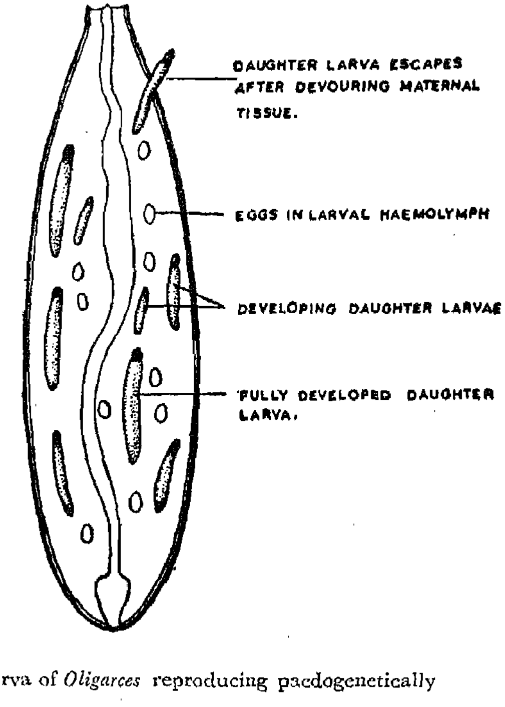
DAUGHTER LARVA ESCAPES AFTER DEVOURING MATERNAL TISSUE.
EGGS IN LARVAL HAEMOLYMPH
DEVELOPING DAUGHTER LARVAE
FULLY DEVELOPED DAUGHTER LARVA.

Fig. 15-13. Larva of *Oligarces* reproducing paedogenetically

Because of the development of larvae in the haemocoele of the mother, this type of reproduction is sometime called as '*haemocoelous paedogenesis*'.

Miaster larvae which under normal conditions develop into usual types of males and females, start giving birth to other larvae as mentioned earlier, if nutritional conditions are either very good or poor. An interesting case of paedogenesis is also found in *Micromalthus* (Coleoptera) in which normal males and females usually occur. Depending upon the environmental conditions, the fertilized eggs either develop into normal adults after metamorphic stages or may change into paedogenetic larvae. Some larvae give rise to several daughter larvae which eventually develop into females. Others produce eggs which adhere to the body of parent larva and when embryo hatches, it eats the parental tissue and develops through a pupa to a normal adult.

Hesperoctenes : It is an external heteropteran parasite on tropical bats, has a rare type of paedogenesis in insects where last instar nymph starts laying fertilized eggs. The fertilization occurs when adult males inseminate the nymphs haemocoelically.

Polyembryony

Polyembryony is the phenomenon where more than one embryo is produced from a single egg which divides repeatedly during the course of its development. It is quite common in a number of parasitic Hymenoptera belonging to the families Chalcididae, Braconidae, Ichneumonidae, Encyrtidae, Platygasteridae *etc.* and in non-parasitic forms like superfamily Vespoidea. In *Platygaster* some eggs produce only one embyro while others undergo a process of polyembryony. In *Ageniaspis* (Encyrtidae), a single egg in the host body undergoes repeated multiplication producing long chain of large number of eggs which are enveloped in an extra embryonic membrane called trophamnion through which nutrients are absorbed from the host tissue. Eggs grow in size and eventually larvae come out and feed on the tissues of the host.

Parthenogenesis

Parthenogenesis is the development of the egg without fertilization and is very widely known to occur in different insects. In some species it is of common occurrence (constant parthenogenesis) while in others it is sporadic (sporadic parthenogenesis). In rare cases the parthenogenetic mode of reproduction alternates with sexual reproduction (cyclic parthenogenesis).

(a) *Sporadic :* Some insect species, under certain physiological conditions, exhibit a tendency towards parthenogenesis. In *Bombyx mori* and *Lymantria* occasionally both males and females are produced from the unfertilized eggs. In Acrididae, the development of embryos proceeds to a great extent even in unfertilized eggs but only in a very few cases they develop into adults. A parthenogenetic strain has been successfully isolated as one of the biotypes or physiological races of the desert locust which reproduced for six generations in the absence of males. The progeny obtained from this race consisted of only females whose development was characterised by delayed growth, sexual maturation and prolonged oviposition. Certain races of *Culex pipiens* also develop parthenogenetically.

(b) *Cyclic* : In some insects like aphids, cecidomyids,. Hymenoptera and others, asexual reproduction cycle is interrupted by the sexual. This is often termed as alternation of generation where the insect derives the advantage of both parthenogenesis and sexual reproduction. In *Aphis fabae* alternation of generations are very complicated. During summer when the conditions are favourable, they reproduce very rapidly on beans by parthenogenesis combining viviparity and paedogenesis. During winter in temperate regions the insects migrate to another host plant (spindle tree) and lay overwintering eggs through the agency of sexuales which are produced during autumn. The eggs overwinter and develop into wingless females which reproduce parthenogenetically and finally give rise to winged forms. They migrate to summer host plants and build up its population parthenogenetically. In tropical countries including India where conditions are more favourable, parthenogenesis predominates in the life cycle of aphids.

(c) *Constant* : In honeybee the males are produced from unfertilized eggs while females from the fertilized ones. Similar situation exists in a number of species of parasitic Hymenoptera and certain coccids. In white-flies the unfertilized female usually give rise only to males but in *Trialeurodes vaporariorum*, there are two distinct reproductive races, one giving rise to males and other to females out of the eggs produced parthenogenetically. The fertilized females, however, produce members of both sexes.

Inhibition

Conditions preventing maturation of gonads : Under certain natural conditions reproductive system is adversely affected so as to inhibit or interfere with the production of ova or sperms. This leads to the castration of the individual. The physiological causes of castration include the nutritional deficiencies which inhibit proper development of the gonads. In honeybee the food of the larval workers is deficient in certain nutrients with the result that the adult worker though female, is non-functional because of undeveloped gonads. Insufficient or imbalanced nutritional conditions adversely affect the endocrine system which is responsible for making the food available to the developing ova in many dipterous and other insects. The adult flies of *Dacus* remain castrated if they fail to obtain in their food a source of hydrolysed protein in an utilizable form. Often parasitisation renders insects sterile which may be so severe that even the secondary sexual characters may be supressed. In certain insects, infection due to nematodes inhibit the growth of gonads and the sex cells fail to develop.

Artificial induction of castration by active gamma rays or by the use of chemicals (chemosterilants) has a great potentiality in insect control. A large population of insects are artificially bred and subjected to irradiation or chemotherapy to render them sterile without adversely affecting their mating or other behaviours. The males are liberated in the field which compete with natural population of males and inseminate females with unviable sperms resulting in heavy reduction in population of succeeding generations.

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16

NERVOUS SYSTEM

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IN a unicellular organism, a single cell performs all the functions of life. There is therefore no need for any special mechanism for coordination of the various activities. In multicellular animals size increases and becomes more complex, its cells transform into different tissues and organs of specific functions. In such an animal, coordination of activities of various organs becomes a necessity for the organism. This coordination is accomplished by the nervous system.

For a proper understanding of the nervous system and appreciation of its role in animal, it is important to know, in general terms, what an animal is made up of and how the activity results and how it is controlled and utilized. We know that every organism is a well organised mass of matter and is composed of many different kinds of chemical compounds. This matter is charged with the potentiality of chemical and physical activities and the latent energy present in it tends to remain in a state of equilibrium with its environment. Different chemical compounds present in an organism are either stable or labile. The stable compounds form the integument, the skeleton, the connective tissue and the supporting frame work of muscles, glands and cells. The labile compounds occur in the secreting cells, glandular cells and the contractile tissues of muscles and the receptive and conductive parts of nerve tissue. The molecular structure of these labile compounds is unstable and cause activity in an animal on receiving a stimulus. Such an activity becomes a necessity to an animal for making suitable adjustments according to changes in its environment. The transmitting of stimulus from stable periphery to internal labile tissues of an organism and the necessary mechanism for controlling and directing the liberated energy for successful living are furnished by the nervous system and its motor mechanism.

Since the conditions immediately surrounding an animal constitute its environment, the environment at tissue and cellular level is different from that at an organism level. The external stimuli *e.g.*, light, smell *etc.*, are not transmitted in kind to the interior of the body. The receptive cells (receptors or sensillae) of the nervous system located on stable periphery are the first to perceive. The stimuli cause change in the metabolic activity of these cells where electrical impulses are generated and then propagated through nerve fibres to the internal tissues. The internal stimuli may be distributed by either inner elaboration of the nervous system or by the production of hormones. The hormones are transported in the circulating medium (haemolymph) and thus initiate activities in tissues and organs far away from their source of origin.

Thus nervous system constitutes the connecting link between the various sensillae responsible for receiving stimuli and the environment on one hand and the effector organs (muscles) on the other. The effector organs respond to stimuli such that the normal functioning of the body goes on smoothly in an organism. In other words, it can be said that nervous system, in general, coordinates various activities in a meaningful way.

The nervous system in insects consists of large number of nerve cells called neurons and non-nervous parts, neural lamella and neuroglia which provide support and nutrition to neurons.

Neuron : The two important properties of protoplasm namely irritability and conductivity are highly developed in neurons. Each neuron consists of a cell body or perikaryon with a nucleus and protoplasmic extensions (Fig. 16-1). The longest protoplasmic extension arising from perikaryon is the axon, while the shorter ones are known as dendrites. Generally the axon gives out a side branch known as collateral, near its origin from the cell body. Axons, collaterals and dendrites distally divide into fine fibrils called terminal arborisations. The dendrites are specialised for the reception of stimuli which are then conducted through the axon.

The neurons may be of monopolar (unipolar), bipolar and multipolar types depending upon the number of protoplasmic projections given out from perikaryon (Fig. 16-2 a, b, c). An aggregation of neurons forms a ganglion and collection of several neuronic strands forms a nerve.

Both the ganglia and nerves are supported by non-nervous neuroglial cells and surrounded by neural lamella. The neural lamella plays an important role in insects by forming a barrier between the potassium ions of haemolymph and the neurons.

On functional basis the neurons are divided into sensory, motor and internuncial or associated neurons.

A diagrammatic representation of the association of all the three neurons in a ganglion and the course of conduction of an impulse are shown in Fig. 16-3.

Sensory neurons : These are also known as afferent neurons and are concerned with the perception of the stimuli and conducting them from the site of perception to central nervous system. The dendritic fibres of the neuron make

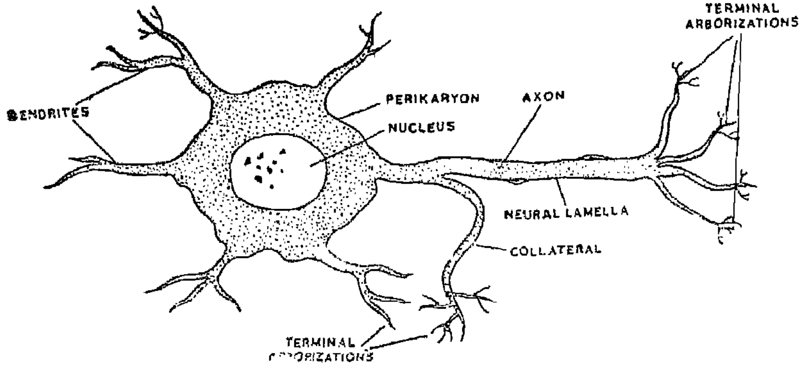

Fig. 16-1. Diagrammatic representation of a neuron.

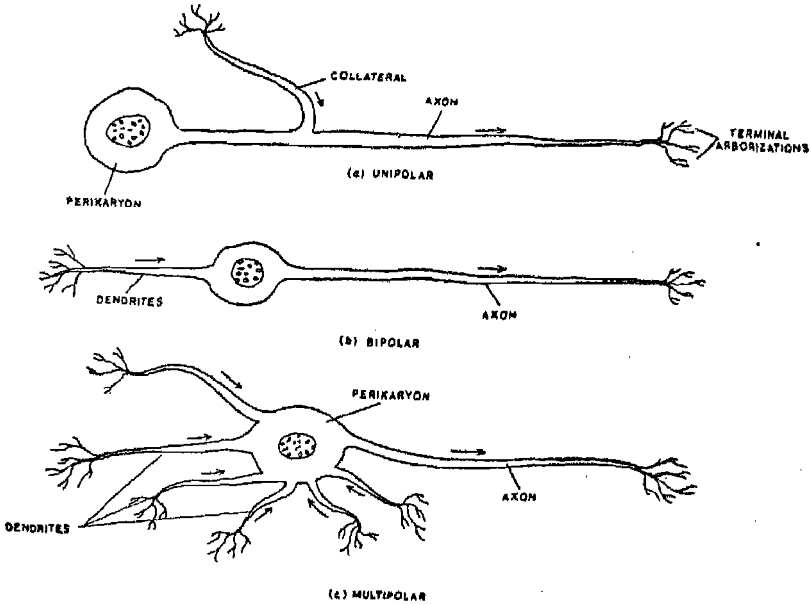

Fig- 16-2· Different types of neurons in insects (arrows indicate direction of conduction)

contact with the sensilla on the cuticle while the axon extends to central nervous system.

Motor neurons : These are also known as efferent neurons

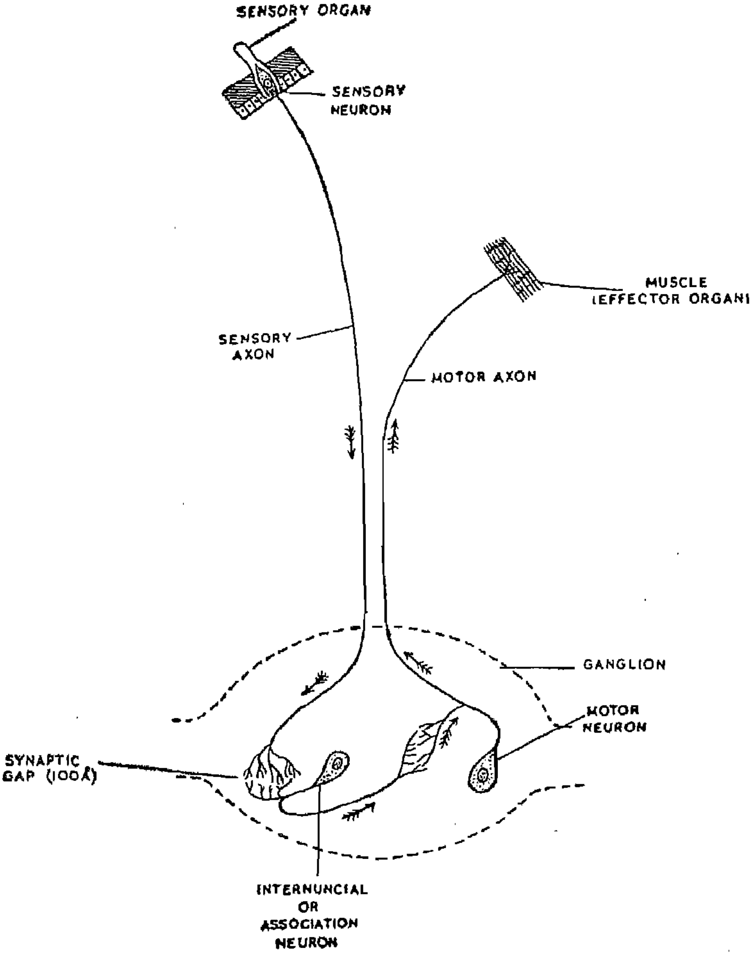

Fig. 16-3. Diagrammatic representation of association of sensory, motor and internuncial neurons in a central ganglion (arrows indicate the direction of impulses).

and are responsible for conducting impulses from the central nervous system to muscles (effector organs).

Internuncial or association neurons. These neurons link the sensory neurons with the motor neurons within central nervous system through synapses.

Synapse. The site at which the axonic end of a neuron comes in close approximation with the dendritic end of an adjacent or a following neuron is known as synapse. Thus there is no direct contact between two neurons at the synapse leaving a gap of about 100 A° (1A° $=10^{-8}$ cm $=0.1$ m μ).

The nervous system of insects can be divided into three parts (i) the central nervous system, (ii) visceral or stomatogastric or sympathetic nervous system and (iii) peripheral nervous system. It must be borne in mind that these are not separate from one another but are inter-connected.

Central nervous system

This forms the major portion of the nervous system and is constituted basically of a pair of ganglia in each body segment united by a transverse strand of nerve fibres known as commissures which are not visible because the two ganglia are very closely united. A pair of longitudinal strand of nerve fibres called connectives join the fused ganglia of each segment with the preceding and following ganglia of the other segments. These connectives extend from the head to eighth abdominal segment connecting various ganglia on the way to form ventral nerve cord. Generally, the connectives are so close to each other that they give an appearance of a single cord. In some insects the connectives are separate throughout the body as in *Machilis* (Thysanura) and *Corydalis* (Neuroptera) while in Orthoptera, Coleoptera and many lepidopterous larvae the separation is clear only in thoracic region. The ganglia of some segments fuse and form the ganglionic centres. Two such ganglionic centres present in the head are the brain and suboesophageal ganglia. The fusion of ganglia to form ganglionic centres in the thorax and abdomen vary from order to order.

The central nervous system can be distinguished into three regions namely (a) the brain or cerebral ganglion or supraoesophageal ganglion, (b) sub-oesophageal ganglion and (c) the ventral nerve cord (Fig. 16-4, 5).

(a) *The brain :* This is one of the two ganglionic centres of the head lying dorsally above the oesophagus and forms the major part of the central nervous system. Being the main association centre of the body it receives stimuli from the sense organs of the head and also from posterior parts of the body through neurons arising from posterior ganglia. It is also the seat of many long term organised behaviour patterns and governs their modification by learning and acts to some extent as a coordinating centre.

The brain is formed due to the coalescence of three pairs of ancestral ganglia of the first, second and third head segments. Despite the fact that it is a fused ganglionic centre, the three fold division is maintained though not always apparent externally and these regions are designated as the protocerebrum, the deutocerebrum and the tritocerebrum.

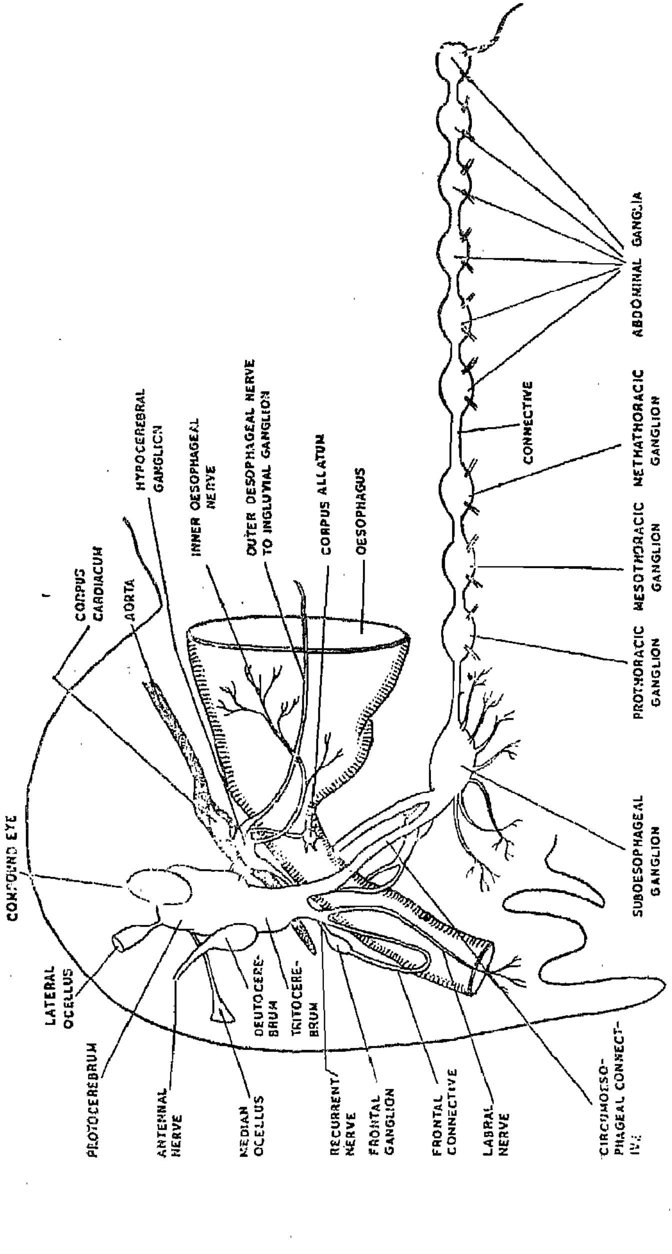

Fig. 16-4. Generalised insect nervous system (lateral view).

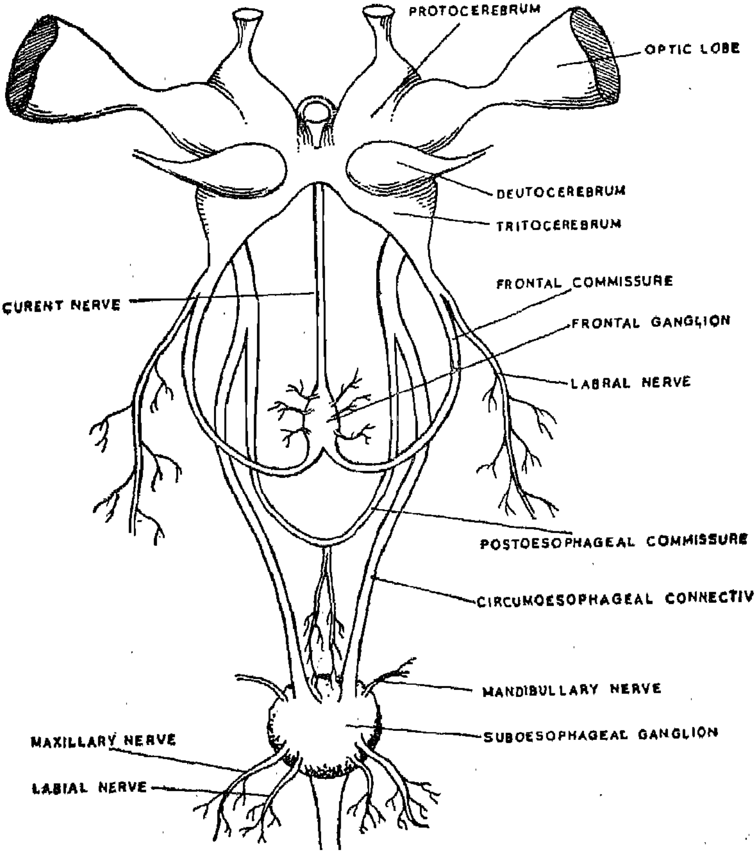

Fig. 16-5. Head-nervous system (anterior view).

The protocerebrum : It is formed due to the fusion of the ganglia of the preantennary or first head segment and forms the greater part of the brain. It is bilobed and is continuous laterally with the optic lobes. The protocerebrum is the most complex part of the brain and supplies nerves to the compound eyes and ocelli.

The deutocerebrum : It lies on the ventral side of the protocerebrum and is also known as mid-brain. The deutocerebrum represents the fused ganglia of the antennary segment and is composed of the paired antennary or olfactory lobes which are divided into dorsal sensory and ventral motor areas. The nerves arising from it innervate antennae.

The tritocerebrum. It is also known as the hind-brain and lies below and behind deutocerebrum. It is formed due to coalescence of the ganglia of the third or intercalary segment of the head and is divided into two separate lobes which are attached to deutocerebrum. The tritocerebral lobe of one side is connected with the other side by a commissure passing behind the oesophagus known as postoesophageal commissure while they are connected to suboesophageal ganglion by circumoesophageal connectives or crura cerebrei (Fig. 16-5).

The tritocerebral lobes are also connected to frontal ganglion of the sympathetic nervous system by frontal commissure. A pair of labral nerves are given out, one from each lobe of the tritocerebrum which innervate the labrum.

(b) *Suboesophageal ganglion.* This is the second ganglionic centre of the head and is formed by the fusion of the ganglia of the mandibular, maxillary and labial segments. It lies below the oesophagus and posterior to the brain. Nerves from this innervate mandibles, maxillae, hypopharynx, labium and neck. As mentioned earlier, the suboesophageal ganglion is connected with the tritocerebrum of the brain by circumoesophageal connectives which encircles the oesophagus. This ganglion, in the case of desert locust, *Schistocerca gregaria* gives off a pair of recurrent nerves and seven pairs of nerves, supplying the mouth parts, cervical muscles, salivary ducts and prothoracic ventral longitudinal muscles.

(c) *Ventral nerve cord.* It consists of the various ganglia lying in thoracic and abdominal region with their connectives and nerves arising from them. Generally, there are three ganglia located in each of the thoracic segments and five abdominal ganglia lying in the abdominal region. The thoracic ganglia are the prothoracic, the mesothoracic and the metathoracic. The prothoracic ganglion is connected with the suboesophageal ganglion by the paired connectives and thus the ventral nerve cord runs from the head to the end of abdomen. The number of nerves that arise from prothoracic ganglion varies. The two principal nerves arising from this ganglion, on either side, innervate the general musculature and leg muscles.

The mesothoracic and metathoracic ganglia give off an additional pair of nerves as compared to prothoracic ganglion which innervate the wings located in the respective segments.

In the desert locust, the mesothoracic ganglion gives off a pair of recurrent nerves and five pairs of nerves innervating hypodermis, fore wings, middle legs, different muscles of the mesothorax and the chordotonal organ. While metathoracic ganglion which is a compound ganglion formed due to the fusion of the first three abdominal ganglia with the metathoracic ganglion gives off five pairs of metathoracic nerves and six pairs of abdominal nerves. The thoracic nerves supply the hind wings, metasternal sensillae, various muscles and hind legs while the abdominal nerves include dorsal and ventral nerves of the first, second and third abdominal segments supplying the abdominal muscles, pleuron, tympanum and spiracles.

The abdominal ganglia are variable in number and the largest number of abdominal ganglia occurring in larval or adult insects is eight as in Thysnura (Fig. 16-6 d), male *Pulex* sp. (Siphonaptera) and many larval forms. The terminal ganglion represents a nerve mass formed by the fusion of the ganglia of the last abdominal segments (Fig. 16-6 a).

Altogether fourteen ganglia were noted from thoracic and abdominal regions, three in thorax and eleven in abdomen in many embryos. The pair in the eleventh abdominal segment fails to appear in some insect embryos. The last three or four pairs of ganglia in this region coalesce into a terminal centre so that in many adults not more than eight or nine ganglia can be counted.

There are five distinct abdominal ganglia in the desert locust. The first four of these give off one dorsal and one ventral nerve which supply the dorsal and ventral muscles in the respective segment. The last abdominal ganglion gives off six pairs of nerves which innervate the muscles of the eighth, ninth, tenth abdominal segments, epiproct, paraproct, cerci and rectum. It also supplies nerves to the complex genital organ of the male and to different valves of the ovipositor of the female. In the female, the ventral nerve of the seventh abdominal ganglion also innervates the oviduct.

The general pattern of the segmental arrangement of ganglia and the innervations suggest segmental autonomy in insects unlike in other higher animals. Regional specialization of the insect body leads to coalescence of segmental ganglia and the shortening of ventral nerve cord. Frequently, one or more abdominal ganglia fuse with metathoracic ganglion as in *Chironomus* and *Schistocerca gregaria* (Fig. 16-6 a). Sometimes all the three thoracic ganglia fuse as in *Stratiomyia* (Fig. 16-6 b). In some families of Diptera and Hemiptera all the thoracic and abdominal ganglia fuse into a single mass located in the thorax *e.g.*, *Musca* (Fig. 16-6 c). The extreme case of centralization of ganglionic mass is observed in scale insects and plant lice where the thoracic mass (formed due to fusion of all thoracic and abdominal ganglia) is coalesced with the suboesophageal ganglionic mass.

Visceral or stomatogastric or sympathetic nervous system

This is associated with the alimentary canal and is divided into (a) oesophageal or stomodaeal sympathetic, (b) ventral sympathetic and (c) caudal sympathetic systems.

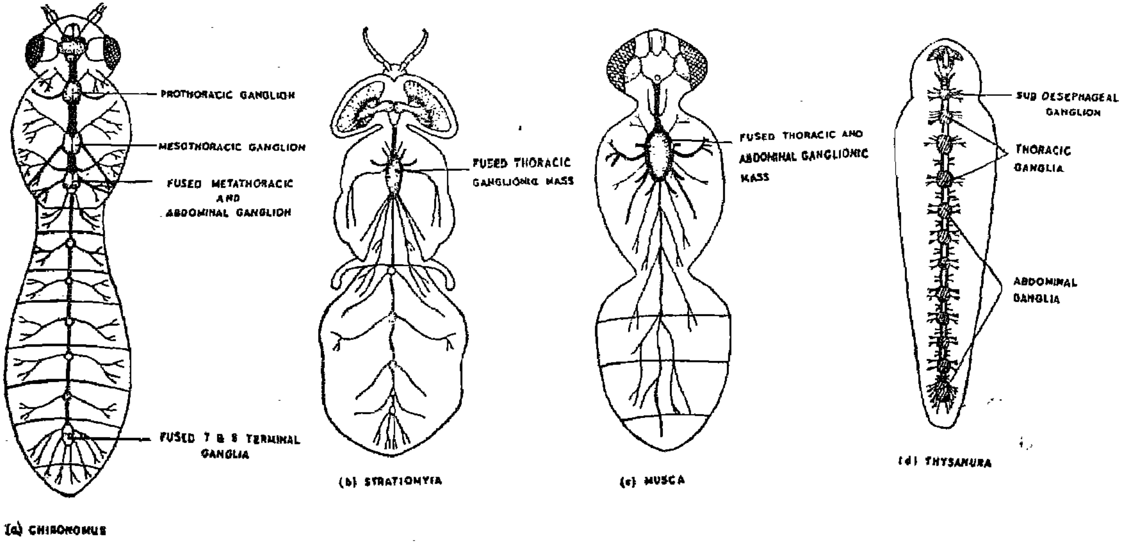

Fig. 16-6. Diagrams showing different degrees of concentration of ganglia.

(a) *Oesophageal or stomatogastric or anterior sympathetic nervous system.* It consists of a median frontal ganglion, hypocerebral ganglion, oesophageal or pharyngeal or occipital ganglion (also called corpus cardiacum) and a paired ingluvial or stomachic (Fig. 16-7). The frontal ganglion is situated above the oesophagus and in front of the brain. This is connected to tritocerebral lobes by a pair of frontal connectives. In some insects, anteriorly the frontal ganglion gives off a frontal nerve which passes to the clypeus, and posteriorly the recurrent nerve which extends along the mid-dorsal line of the oesophagus passing beneath the brain and connects the hypocerebral ganglion. From hypocerebral ganglion nerves go to *corpora cardiaca* or oesophageal ganglia of the stomodaeum and the ingluvial or stomachic ganglion situated on the posterior end of the foregut. Nerves from the ingluvial ganglion innervates the neighbouring regions of the stomodaeum.

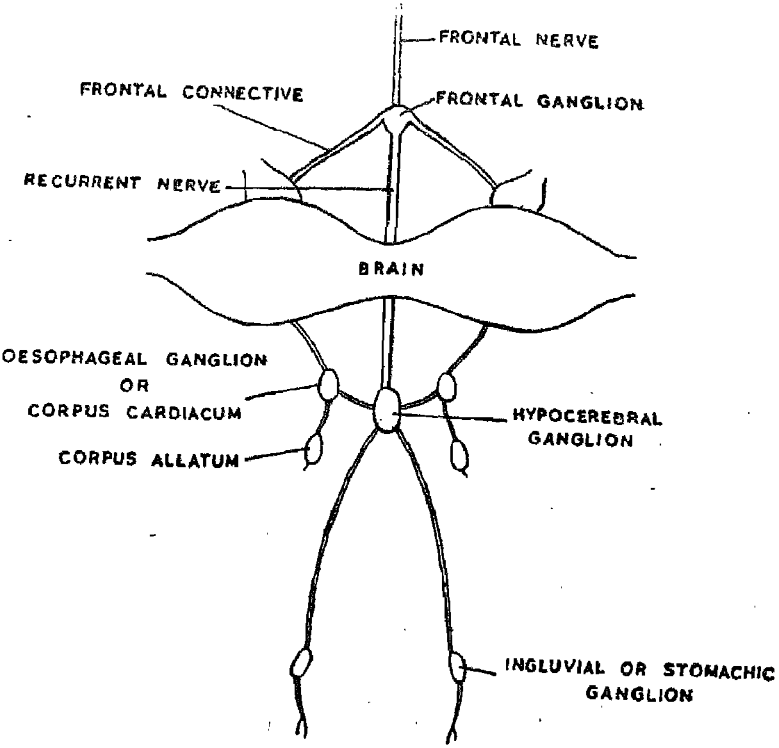

Fig. 16-7. Sympathetic nervous system of an insect.

There may be variations from the generalized Pterygote plan mentioned above. For example, in the larvae of cyclorraphan Diptera, the hypopharyngeal ganglia, corpora cardiaca, corpora allata fuse to form a composite structure known as Weissmann's ring which encircles the aorta just behind the brain. In the Apterygota, the stomatogastric system is variable usually reduced but, however, the frontal ganglion is always present.

The frontal ganglion may be responsible for controlling swallowing movements. In *Locusta,* and probably in other insects, it may be concerned in controlling the release of secretion by the corpora cardiaca. Ingluvial ganglion directly controls peristaltic movements of the fore and midgut.

(b) *The ventral sympathetic nervous system.* It consists of a single median nerve and a pair of transverse nerves associated with each ganglion (Fig. 16-8) of the ventral nerve cord.

(c) *The caudal sympathetic nervous system.* This consists of the nerves arising from the posterior ganglion of the abdominal nerve cord and innervating the reproductive system and posterior part of the gut.

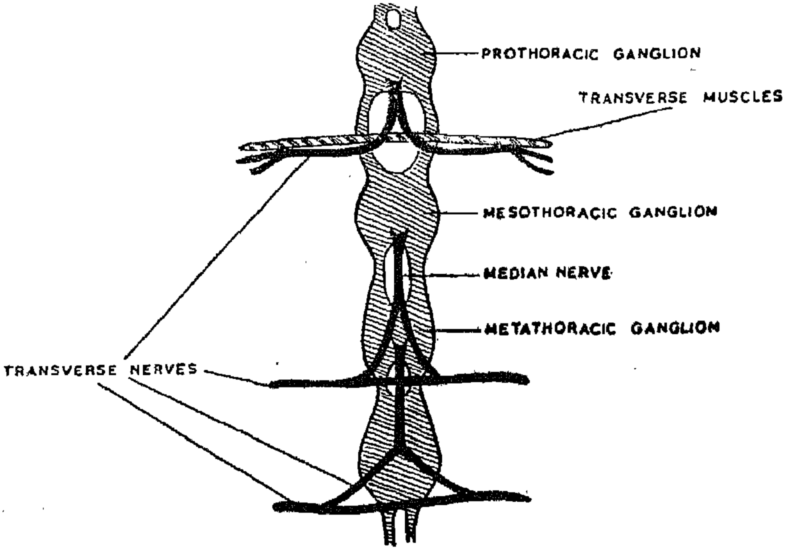

Fig. 16-8. Ventral sympathetic nervous system.

Peripheral nervous system

It consists of nerves radiating from the ganglia of central and sympathetic systems. The axons of the sensory neurons of receptors run from periphery of the insect inwards to central nervous system while the axon of motor neurons go outward to muscles and glands from central nervous system. Thus this includes the nerve trunks radiating from the ganglia, and the distal branches and terminal organs of the motor and sensory fibres contained in the nerve trunks.

The peripheral sensory nervous system present in insects is a delicate plexus formed by bipolar and multipolar sensory neurons.

Conduction of nerve impulse in axons

Any change that brings an alternation in metabolic activity of the sensitive constituents of living matter is called a stimulus. The protoplasm being sensitive to these stimuli, transmits the change from molecule to molecule and this property of progressive reaction to stimuli is known as *conductivity*. The time required for the restoration of the original condition of the system after the change is called the *recovery period*. *Fatigue* results when stimuli follow in too rapid succession to allow of complete recovery.

The various stimuli perceived through sensillae distributed all over the body are conducted to the central nervous system through the axonic fibres of sensory neurons and the response to these stimuli is transmitted to effector organs (muscles) through motor neurons. Whatever be the type of stimulus, whether mechanical, chemical or visual and irrespective of the method of perception, the energy received by the sense cell is transformed to electrical energy. It is now known that the conduction of impulse among the axon is an electrical phenomenon and it has been clearly shown that electrical waves pass through the membrane of the axon during the transmission of impulses. However, the conduction of impulse at synapse is a biochemical phenomenon and is discussed elsewhere (Chapter 17).

In the resting condition of the membrane, there exists an ionic imbalance between inside and outside the axon despite the fact that the plasma membrane of the axon is a freely permeable. This is due to the role of sodium-potassium pump. Sodium ions (Na^+) predominate the outer region of the axon whereas potassium ions (K^+) are 24 times greater inside the axon as compared to outside. The greater amount of potassium ions inside imparts a negative charge on the inner surface of the axon (Fig. 16-9) and the predominance of the sodium ions outside charges the outer surface of the membrane positive. The electric potential produced in this way is known as the *membrane or resting potential*. The magnitude of this potential in insect nerve axon varies and in general is about —70 mV with reference to the membrane outside.

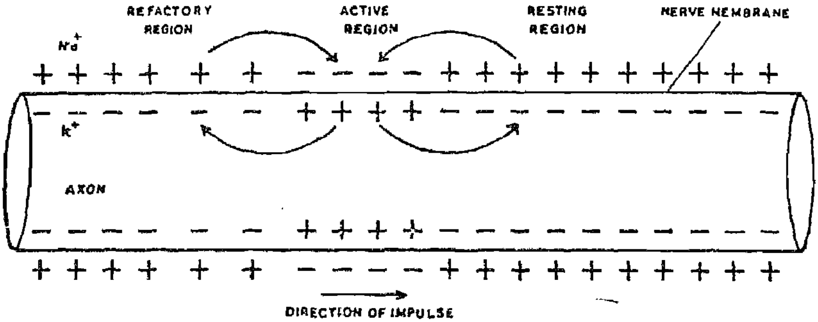

Fig. 16-9. Diagram of impulse conduction in an axon (arrows indicate current flow in local circuit).

We have already seen that in a resting condition the membrane of an axon is positively charged outside and negatively charged inside and is in a polarised state.

When an impulse reaches a point on the membrane, the permeability to Na+ increases tremendously resulting in an inward movement of Na+ and an outward flow of K+. This leads to a positive charge inside the membrane as compared to outside amounting to 80-100 mV in the cockroach and the membrane is said to be depolarised. At this stage the adjacent areas, with reference to the point of depolarisation (*i.e.*, active region) are negatively charged, such that a current flows in a local circuit away from the point of depolarisation inside the axon and towards it on the outside. This current flow away from the point of depolarisation inside the axon leads to depolarisation in the areas of resting condition where it reaches. Thus, the nerve impulse is propagated along the axon due to a wave of depolarisation leading to greater permeability of the membrane.

The various phases of action potential during the transmission of a nerve impulse across the plasma membrane of an axon are shown in Fig. 16-10. In resting condition, the membrane potential is of the order of —70 mV with reference to outside the membrane. The depolarisation of the membrane, due to a stimulus, leads to greater influx of sodium and forms the rising phase of the action potential. After the rising phase, the permeability of the membrane increases to potassium resulting in a greater influx of potassium and the charge of the inside membrane now changes to negative. This constitutes the falling phase of the action potential. The total duration of spike or action potential is very short and may be of 1 or 2 milliseconds. Due to greater inflow of K+ the potential not only returns to the resting condition of the

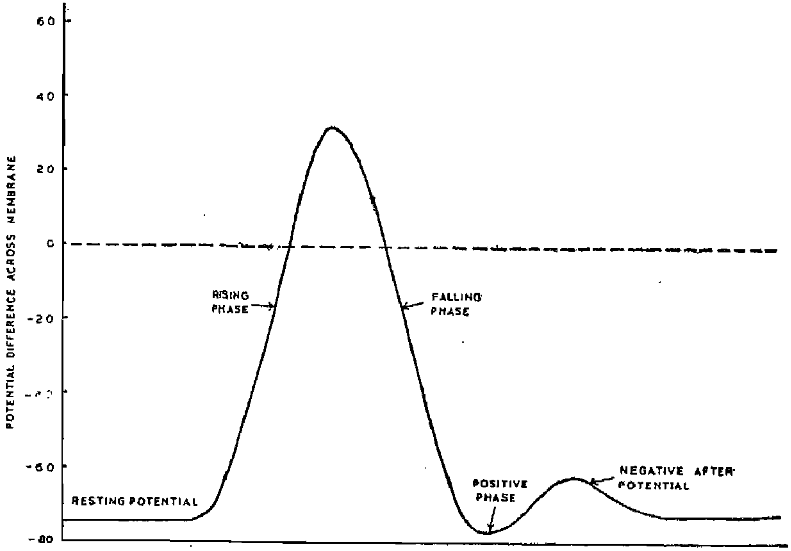

Fig. 16-10. Diagram of the changes in potential difference across the plasma membrane of an axon occurring during the passage of an impulse (after chapman).

membrane but also over it, resulting in the positive phase of the action potential. After the positive phase, the potential swings back again to a level slightly higher than normal and forms the negative after potential phase. The phase of negative after potential results due to the accumulation of K^+ outside the membrane during the falling phase of the action potential and this accumulation reduces the tendency of the movement of K^+ outside. The membrane returns to its original (resting condition) from the negative after potential phase due to sodium pump which actively extrudes Na^+ in exchange for K^+.

The stimulation of nerve, irrespective of stimulus, leads to many impulses. Since the amplitude of the impulse from the various stimuli is more or less the same, a question arises how the information pertaining to these stimuli is conveyed ? Probably, it is the number and frequency of impulses that convey the information.

Sense organs or receptors

Nervous system acquaints the insect with changes in its environment by means of a variety of receptors located all over the body. These sense organs not only detect and interpret these changes but also translate them into nerve impulses that ultimately produce responses in muscles or glands thereby helping the insect in adjusting to the changed conditions. Thus these act as mediators between the environment and the nervous system.

Sensory receptors may be external (eyes, antennae, palpi and setae) or internal (proprioceptors and the sympathetic receptors). External stimuli are perceived by exteroceptors while interoceptors react to internal stimuli.

Essentially a sense organ or sensillum consists of a cuticular or hypodermal structure and one or more sensory neurons associated with it (Fig. 16-11). The sensory neuron with its dendritic end is in contact with the cuticular structures and its axonic end terminates in the central nervous system. The cuticular structure with the sensory dendron serves as a receptor for perceiving stimuli and also facilitates somehow to get the stimulus transformed into an impulse in the dendritic fibre. The impulse once generated is then conveyed along the axon to central nervous system.

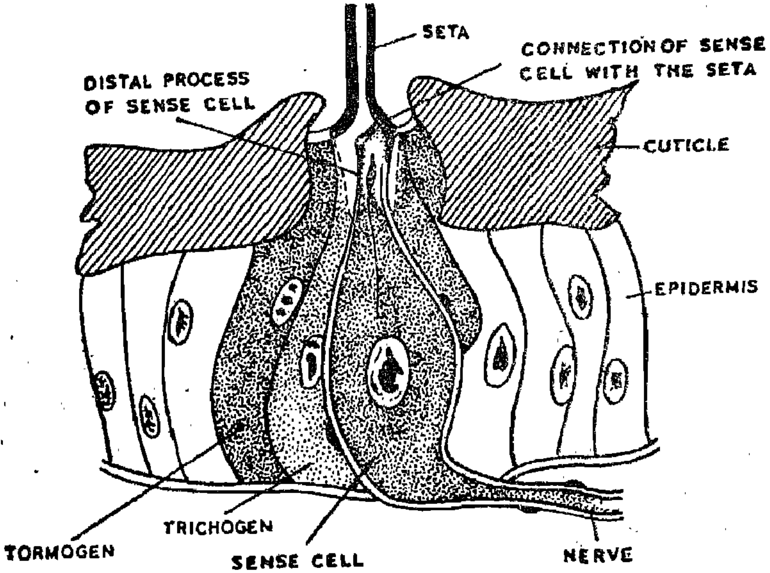
SETA
CONNECTION OF SENSE CELL WITH THE SETA
DISTAL PROCESS OF SENSE CELL
CUTICLE
EPIDERMIS
TRICHOGEN
TORMOGEN
SENSE CELL
NERVE

Fig. 16-11. Generalized type of sensillum.

The cuticular structures are of varied types and differ from one sensillum to another. Some sense organs have an external process, the seta which is modified into different shapes. The sense organs having spine-like setae are known as *sensilla chaetica* which are tactile in function while those

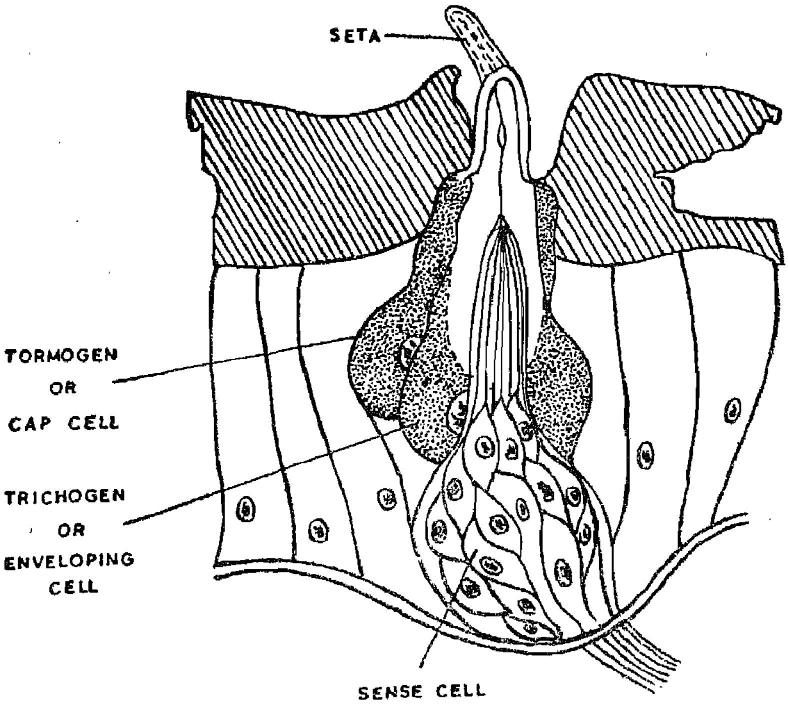

Fig. 16-12. Sensillum basiconicum.

with peg or cone-like setae are the *sensilla basiconica* (Fig. 16-12). The *sensilla basiconica* are generally regarded as receptors of taste or odour (chemoreceptors). In these sensilla, the external cuticular processes are very thin walled and are permeable to solutions. Besides the above, other sense organs in this category are *sensilla trichodea* (sensory hairs), *sensilla squamiformi* (tactile in function) and *sensilla coeloconica etc.*

Some sense organs do not carry any external structures as mentioned above. In such organs sometimes the cuticle thickens and appears like a dome or bell-like structure in a cross section surrounding the distal process of the sense cell. These are termed *sensilla campaniform* (Fig. 16-13) and occur on the head, thorax, abdomen, antennae, mouth parts, legs, wing bases, cerci and ovipositors of adults of many insects. These receptors may respond either to direct or to the stresses of body movement. Some believe that they are involved in olfaction. In some sense organs under this category the distal process of the sense cell is covered by an oval or elliptical

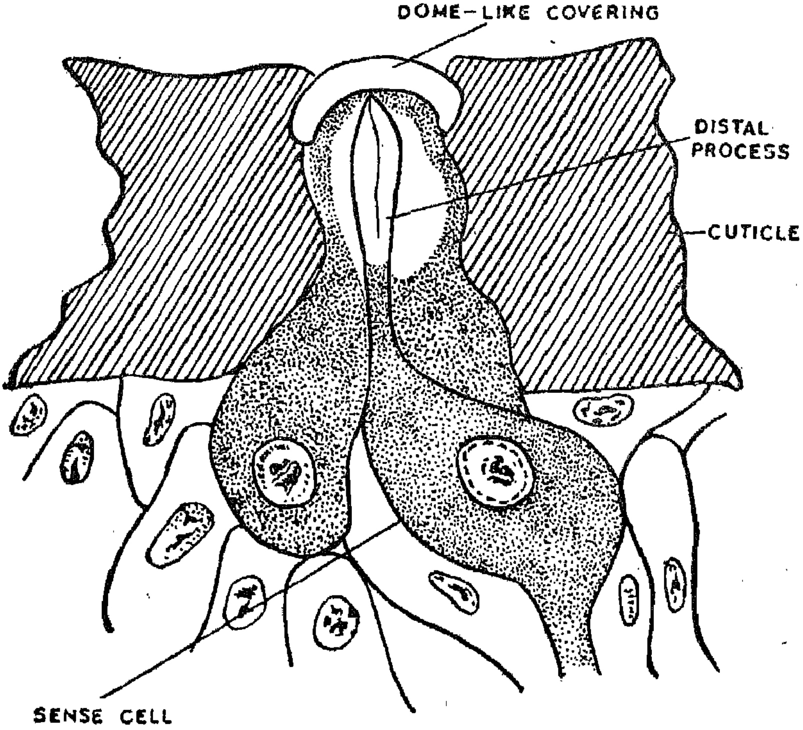

Fig. 16-13. Sensillum campaniform.

outer plate forming a ring of membrane and these are known as plate organs or *sensilla placodea* (Fig. 16-14). These are usually olfactory in function.

The rod bearing sense organs are known as *sensilla scolopophora* (Fig. 16-15). Each sense cell in this sense organ is associated with two other cells *viz.,* cap cell and enveloping cell and its distal process ends in a rod-like structure, the scolope or scolopale. These sense organs may act as proprioceptors and also as auditory receptors.

Distinct from all the above, the eyes form a special group of sensory receptors, the *sensilla optica* (Fig. 16-16). In this type of sense organs, the cuticle forms a transparent area (cornea) that admits light to the receptive sense cells below. These sense cells form the retina and their axons form the optic nerve.

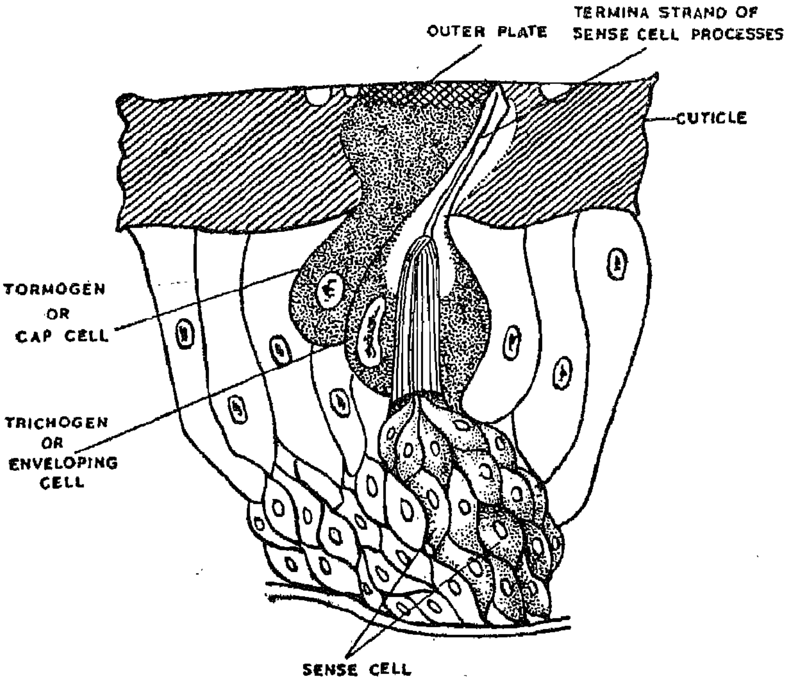
TERMINA STRAND OF SENSE CELL PROCESSES
OUTER PLATE
CUTICLE
TORMOGEN OR CAP CELL
TRICHOGEN OR ENVELOPING CELL
SENSE CELL

Fig. 16-14. Sensillum placodeum.

Depending upon their specific responses to the stimuli, the insect sense organs may be classified as following:

Type of receptor	*Responsive to*
(a) Mechanoreceptors	Pressure or contact
(b) Audioreceptors (auditory organs)	Sound
(c) Chemoreceptors	Chemical in solutions or gas
(d) Photo-receptors (Visual organs)	Light
(e) Thermoreceptors	Temperature
(f) Hygroreceptors	Humidity
(g) Proprioceptors	Body movement, pressure on internal organs like intestine

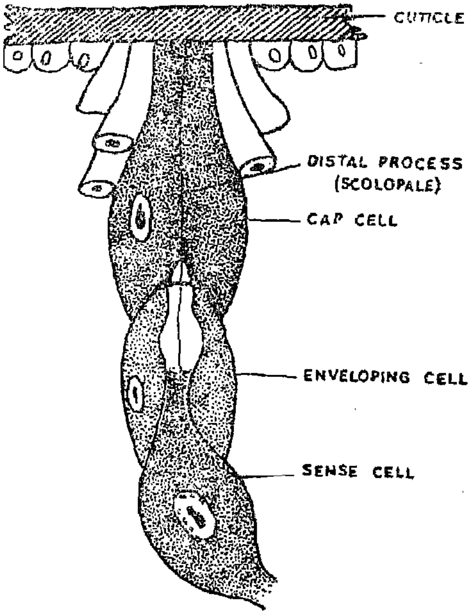

Fig. 16-15. Scolopophorous sensillum.

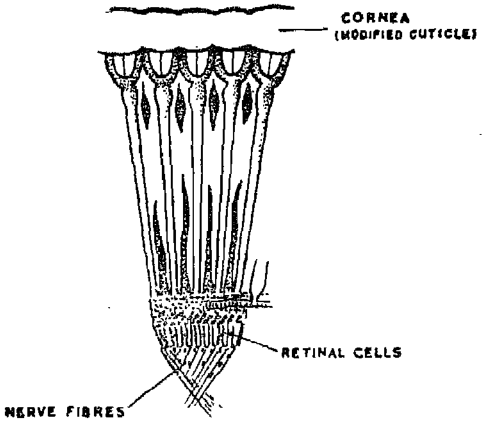

Fig. 16-16. Diagrammatic representation of a part of compound eye.

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17

BIOCHEMICAL BASIS OF NERVE TRANSMISSION IN INSECTS

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THE two fundamental properties of protoplasm which reach their highest development in the nervous system are irritability and conductivity. Possessing such a system, the animal body can quickly respond to stimuli arising from environmental changes and can transmit impulses to the various parts of the body, so that proper adjustments necessary for life can be made.

The general function of the nervous system is integration which means that various responses are so integrated as to act towards a common end. The parts of the body must act as a unified whole if life is to continue. In the body, the nervous system functions as a mechanism for directing the activities of the various parts according to the needs of the whole body.

The importance of the study of neurophysiology of insects can be two-fold:

(i) Insects present a variety to test organisms in terms of anatomy, morphology, physiology *etc.*, which provide an opportunity for study of comparative physiology.

(ii) As most of the newer insecticides are neurotoxicants, an understanding of the basic biochemical phenomena of the nervous system would be highly rewarding.

General aspects

Even though there is a close analogy between electrical conduction and neural transmission, these differ in two aspects.

(i) The velocity of impulse transmission in the nervous system is very low compared with that of electricity. The speed of electricity is much higher depending upon the nature of conductors. The average speed of nerve impulses in animals ranges only between 1 to 100 metres/second. It is 5 metres/second in the case of insects and 100 metres/second in man.

(ii) Electricity can pass through even dead materials while nerve impulse cannot.

Any change in the environment which can cause excitation in the nervous tissue forms a stimulus. It can be of varied nature: electrical, mechanical, thermal or chemical.

Larger the diameter of the nerve fibre, the faster the impulse travels through it. Conduction velocity also depends very much on the temperature; the higher temperature, the faster is the velociy. For most nerves, the relationship is simple and linear over a range of at least 10°C, the rate being approximately doubled by 10 degrees rise.

The amount of response a cell produces is essentially independent of the size of the stimulus. Strong stimuli do not produce a larger response from it than weak ones because every cell, however excited, exerts itself to the maximum of its capacity.

Cellular aspects of the nervous system

Neuron : The nervous system is composed of excessively elongated cells which transmit impulses from one part of the body to another. These nerve cells or neurons, are derived in the course of development from the ectoderm. Each consists of a nucleated cell body and a long filament called *axon*. The axon generally gives off a lateral branch or *collateral* near its origin and both axon and collateral end in fine branching fibrils, the *terminal arborization*. Similar fibrils arising from the nerve cell body form the *dendrites*. Where the bundles of axon filaments run freely through the body, these constitute the nerves.

The sensory neurons (afferent) convey impulses inwards from the sense organs; motor (efferent) neurons conduct them outwards to the muscles; and association neurons link the sensory and motor neurons together within the central nervous system.

Nerve sheath : It has two layers—an outer non-cellular layer *neural lamella* and an inner cellular layer *perineurium*. Perineurium cells contain abundant mitochondria and serve as a store house of glycogen. These cells also help in the diffiusion of nutrients from the surrounding fat bodies and haemolymph.

Synapse : The neurons are not continuous with one another. The branched terminals of the axon of one neuron come in intimate association with the collateral or terminal arborizations of another neuron to form a synapse. Smith and Treherne (1963) measured the gap between adjacent neural terminal and found to be equal to 100 A°.

Nerve-muscle junction : The cellular sheath that invests the peripheral axons along their course from the central nervous system is absent at least on one side of the terminating nerve branch, allowing the axon plasma membrane to lie very close to that of the underlying muscle fibre. A narrow gap is always retained between these two membranes: this gap being about 400-500 A° wide in vertebrate junctions, of as little as 100 A° wide in those of insect muscles.

Conduction in axon

It has been clearly shown that electrical waves pass through the membrane of the axon during the transmission of impulses. This concept forms the very basis of the *membrane theory* of axonic conduction. During the resting stage, there is ionic imbalance between the outer and inner regions of the axon. Na+ predominate the outer part, while K+ are about 20 times more inside than the outside. The ionic imbalance creates bioelectricity which can be measured using a galvanometer. At this stage the membrane is said to be *polarized* and the outside of the membrane is positively charged with respect to the inside. The giant axon of *Periplaneta* has a resting potential of about 77 mV.

While the nerve becomes active *i.e.*, as the impulse reaches a point on the membrane, the permeability of the membrane instantly increases, where upon Na+ rush inside destroying the customary concentration gradient. At the instant when the movement of Na+ reaches the peak, K+ begin to pour in the opposite direction. During the stage, the membrane is said to be *depolarized* and a reversal of electrical charges occurs.

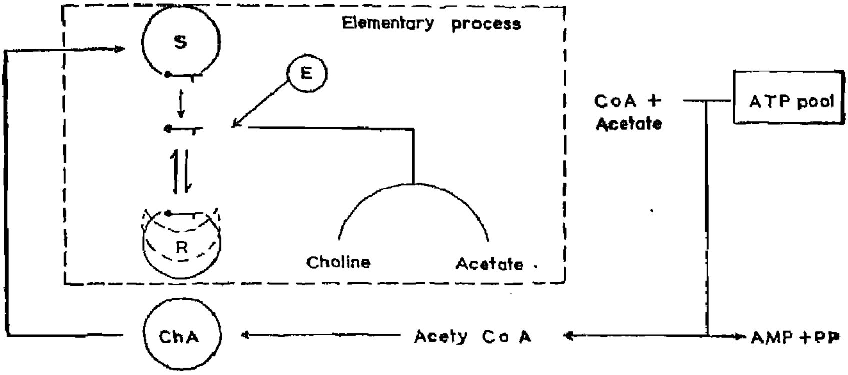
The current produced by the rapid in and out movement of the K+ at a particular area of the membrane is sufficient to depolarize the contiguous regions of the membrane and the impulse passes on forward.

It is difficult to see how electricity in a fluid system such as the living cell can be generated without chemical reactions. Conducting cells must be endowed with a special chemical system controlling the movements in a specific way. The rapid development of biochemistry, especially of protein and enzyme chemistry during the last three decades, has provided powerful tools for analysing cellular function in terms of physics and chemistry. The enzymes affecting hydrolysis and formation of acetylcholine (ACh) were analysed, the sequence of energy transformations was established and a number of chemical reactions were correlated with physical events.

In the resting condition, ACh is in a bound and inactive form. It may be tentatively called the storage protein (S). Excitation of the membrane leads to a dissociation of the complex and ACh is released. The free ester acts upon a receptor protein (R), and this action upon the receptor is essential for the change of membrane permeability.

The complex between ACh and the receptor is in a dynamic equilibrium with the free ester and the receptor. The free ester is susceptible to attack by cholinesterase (E). The enzymatic hydrolysis of ACh will permit the receptor to return to its resting condition. Thus the action of the enzyme leads to immediate recovery and ends the cycle of elementary

The picture of the elementary process which has emerged from these studies may be described as follows:



S=Storage protein E=Cholinesterase
R=Receptor protein •┬ = ACh.

process. The further recovery leads to the resynthesis of ACh in its bound form by cholineacetylase (ChA) and the other components of the acetylating system.

Synaptic conduction

The biochemical mode of conduction at the synaptic region between the two nerve endings is almost unquestioned. The current concept of cholinergic transmission includes (i) the arrival of the electrical action potential at the axon terminal, causing (ii) the liberation of ACh vesicles, which diffuse across the synaptic cleft and (iii) combine with a receptor-protein to bring about a localized depolarization. Largely on the basis of the histochemical and pharmacological studies in vertebrate synapse, an additional step has been proposed. The immediate action of the liberated ACh on the presynaptic terminal, causes it to release additional quanta of ACh in order to facilitate transmission across the cleft. This would serve as an amplification mechanism at the terminal. It has been suggested that the major function of the localized acetyl cholinesterase (AChE) is to prevent the perpetuation of this process of self-excitation.

Once the impulse has been triggered at the post-synaptic region, ACh has to be broken down to acetic acid and choline. This reaction is mediated by the enzyme, AChE. ACh is resynthesized in the presence of another enzyme, cholineacetylase and the actual mechanism of synthesis will be discussed later.

Conduction at neuromuscular junction

ACh is the transmitter at the neuromuscular junction in vertebrates. But no ACh has so far been detected at the neuromuscular junction of insects. Recent studies have shown that l-glutamate may be the neuromuscular transmitter in insects (Usherwood and Machili, 1968). Holman and Cook (1970) isolated l-glutamate and l-aspartate from the neuromuscular junction of the hindgut of the cockroach and showed their transmitting properties.

Components of cholinergic system

Acetylcholine (ACh): The neurotransmission properties of ACh (β-acetoxyethyl trimethyl ammonium hydroxide) were brought to light through the classical experiments of Loewi (1921). Following the experiments of Loewi, an overwhelming number of investigations have been carried out to establish the significance of ACh in nervous and neuromuscular activity.

The first report of the presence of ACh in insects came from Gautrelet (1938) for bees and from Corteggiani and Serfaty (1939) for eight different species of insects. Mikalonis and Brown (1941) gave the physiological function of ACh in insects through a remarkable series of experiments. But the occurrence of ACh in insects was doubted by Prosser (1952) who pointed out that the nervous tissue of cockroaches contained only an analogue of ACh.

Lewis (1933) obtained evidence by the use of partition chromatography that two species of blowflies contained ACh. But the most conclusive chemical and pharmacological evidences were provided by Mehrotra (1960) who showed that the developing embryos of the milkweed bug and housefly contained acetylcholine.

Cholinesterase (ChE): ChE in insects was first reported by Gautrelet (1938), who found a high titre of ChE and ACh in the heads of bees. ChE has since been demonstrated in numerous hexapods and in other arthropods as well.

ChE often is absent even from nervous tissue in the very young insect embryo. The pattern of enzyme activity during the post-embryonic period in *Chilo zonellus* has been reported by Singh and Mehrotra (1970). ChE is widely distributed in the adult insect and mite nervous system.

The location of ChE in the central nervous system in relation to ACh is also by no means certain. Although the available data are not sufficiently refined to indicate more than this there is considerable overlap in the distribution of the two materials. It is obvious that much of the neuronal substrate must normally be kept from contact with the enzyme. One possibility is that ACh within the tissues, although close to the enzyme, is held there in a bound form that is not readily hydrolyzable. Or again, the ACh may be separated from the ChE by structural barriers.

The ChE in insects exists in homogenates both in soluble and insoluble forms. The pH optimum for fly head ChE was found to be at least as high as 8.0 (Chadwick *et al.*, 1954). Enzyme activity is directly proportional to temperature within certain range of temperatures beyond which heat inactivation occurs. ChE of insects, like those of many other animals, are relatively stable, at least in the impure state.

The attempt to obtain a concentrate of insect ChE in the form of an acetone powder has frequently been unsuccessful. Dauterman *et al.*, (1962) have obtained 157-fold purification of fly head ChE by means of ammonium sulphate fractionation and calcium phosphate gel adsorption. There are differing reports on the molecular weight of ChE. Dale and Mehrotra (1971) have shown by gel filtration technique that ChE exists in different polymeric forms and that the basic monomer unit has a molecular weight of 82,000.

Cholineacetylase (ChA): The elucidation of the probable mechanism of ACh synthesis in insects can be attributed to the clear and concise study of Smallman (1956) with heads of blowfly. In insects, as in other organisms, synthesis of ACh *in vitro* is a two-step mechanism.

$$\text{ATP} + \text{CoA} + \text{acetate} \rightleftharpoons \text{acetyl CoA} + \text{AMP} + \text{pyrophosphate}$$
$$\text{Acetyl CoA} + \text{choline} \longrightarrow \text{ACh} + \text{CoA}$$

Cholineacetylase is the enzyme catalysing the second reaction.

By ammonium sulphate precipitation techniques Mehrotra (1961) purified ChA twenty-fold from extracts of housefly heads. The Michaelis-Menton constant (Km) of the enzyme for acetyl CoA was 4.6×10^{-4}M.

Receptor protein: A receptor was postulated by Langley as early as 1907. Its existence as a cell constituent distinct from AChE has now been experimentally demonstrated. Chagas *et al.*, (1958) were the first to isolate this protein.

Recently, O'Brien and Gilmour (1969) used (3H) muscarone, a compound similar in structure to ACh. It is not hydrolysed by ChE, for which it has a relatively low affinity. The workers could show that muscarone was bound to a particulate fraction in the way that would be expected for binding to an ACh receptor in terms of degree of binding and affinity. Eldefrawi and O'Brien (1970) extended the studies using housefly brain homogenate. The supernatent fluid fraction isolated from a homogenate of housefly heads, bound muscarone reversibly. The binding appeared to involve ACh receptor.

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18

ENDOCRINE SYSTEM

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INSECTS are fascinating animals and the ideal for physiological investigations. These are quite often studied not because these are insects but because different aspects of physiology can easily be studied in them. In the understanding of endocrine mechanism insects play a key role. In general, insect endocrine system is simpler and produces few hormones yet the information derived from them about the fundamental mechanisms of hormones are quite valuable. Insect endocrine system has four major components: groups of neurosecretory cells in the brain (NSC); the corpora cardiaca (CC); the corpora allata (CA); and the prothoracic glands or their homologues (PG). The cerebral neurosecretory cells and the corpora cardiaca form a neurosecretory system (Fig. 18-1).

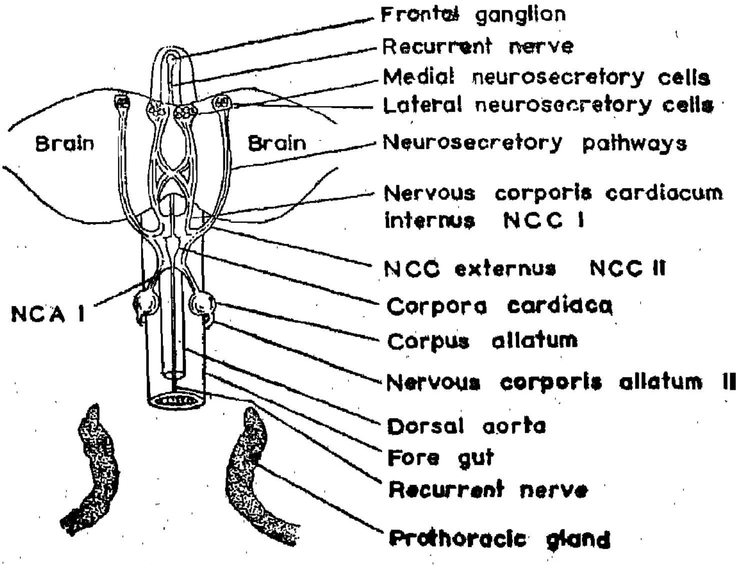

Fig. 18-1. Endocrine system in a generalized insect.

Neurosecretory organs

During the past couple of decades certain staining techniques notably Gomori's chromealum-heamatoxylin-phloxin (CHP) and its modifications; Gomori's aldehyde-fuchsin (AF) and modifications; Adams and Sloper's perfomic acid-alacian-blue (PAAB), and its modification—the performic acid-victoria blue (PAVB), proved a useful tool for the demonstration of neurosecretory system in insects. Recently, a different type of neurosecretion which stains intensely with Azan but is CHP and AF-negative, has been discovered.

Neurosecretory system is located in part at least, deep into the nervous system. This makes it difficult to study its gross morphology by simple dissections. The usual way of demonstration of neurosecretory system was routine histological technique and morphological picture obtained through the reconstruction of serial sections is indirect, unduly lengthy and quite often erroneous. Loss of one or more sections during staining made the series useless. These difficulties led to the development of *in situ* method of staining the neurosecretory system. This method is quick, economic and results obtained provide a direct picture of the neurosecretory system. There is an added advantage that bulk stained material can be used for histological details.

It is of further interest that neurosecretory system revealed in sections or in mounts is mainly due to the stainable material present in the system at the time of fixation. During active physiology, when the demand is more than the supply, there is little or no visible material present in the system and a study of which may provide an incomplete picture. It has been experienced that study of the system at static physiological stages or after inhibiting the system by starvation or by the nerve inhibitors, or by saline injections so as to retain their diuretic principles, undoubtedly provide by far a superior morphological picture of the neurosecretory system.

The neurosecretory cells: The neurosecretory cells have been defined as neurons which also show cytological characteristics of gland cells. These receive nervous impulses which are not transmitted to other neurons or effector cells, instead the neurosecretory axons terminate in close proximity of circulatory system and release their substances in the blood which act on distant effector organs or on other endocrine glands.

In a generalized insect, the cerebral neurosecretory cells are usually in two groups in each half of the pars intercerebralis, part of the protocerebrum. The medial groups are often closely apposed; most of their axons decussate in the brain before passing in the corpora cardiaca (neurohaemal organ). The lateral groups are smaller than the medial groups and their axons run directly into the ipsilateral corpus cardiacum. The corpora cardiaca thus receive inner and outer nerves (nervi corporis cardiaci I and II) from the neurosecretory cells. In insects the number of neurosecretory cells vary greatly; in *Bombyx* there are only 4 cells in each group while in *Schistocerca* about 600 cells in each group. The staining reactions of the two cerebral neurosecretory cells allow the differentiation into main types, A- and B- cells. A-cells stain with the basic dyes of these stains and B-cells with the acid dyes; later on two more types C- and D-cells were also recognized. Experimental and ultrastructural studies carried on so far favours the possibility of more than one type of neurosecretion.

Neurosecretory cells have frequently been reported from the suboesophageal and ventral nerve cord ganglia of the central nervous system. These store their neurosecretion into a series of small neurohaemal organs called as 'perisympathetic organs' found in the vicinity of the small median ventral nerve. These have been suggested to play some role in diuresis, diurnal rhythm and pupal diapause.

Synthesis of neurosecretory material (NSM) : NSM seems to be synthesized exclusively in the perikaryon of the cells, although there is evidence that hormone assemblage may take place along the neuroseretory axon. The raw material of the synthesis of NSM is synthesized in the endoplasmic reticulum and it is packaged by the golgi apparatus and is finally punched off from there as neurosecretory granules which are electron dense-spheres between 1000 to 3000 A^0 in diameter, surrounded by a thin membrane. The protein material synthesized by the neurosecretory cells, called as 'neurophysine', is considered to be carrier for the actual hormone.

Transport of NSM : The synthesized material is transported along the axons of neurosecretory cells. The direct proof of transport of material is provided by nerve sectioning and ligaturing experiments. NSM is thought to be transported by the proximodistal 'flow' of the axoplasm. Little information is available on the transport of NSM in insects. Experiments of Paul Weiss with the neurons suggested a continuous secretion of axoplasm by the cell body of a neuron at a rate of 1 mm/day. Direct observation on the movement of NSM in vertebrates suggest a rate of 100-200 mμ/min. Much more information about the rate of transport of NSM is needed.

Storage and release of NSM : Neurosecretory material of stored for a longer or shorter time in the neurohaemal organs and from there it is released into the blood. The material is actually stored in the swollen axon terminals and from there it is directly released into the blood. The neurohaemal organs mostly provide a supporting framework to the neurosecretory axons. The largest nerve endings observed in the neurohaemal organs are over 5μ in diameter containing secretory granules, 1500 to 3000 A^0 in diameter. The storage area for the NSM is increased by profuse branching of the neurosecretory axons. It is not clearly known how the neurosecretion would actually reach outside of the cell. A number of working hypotheses have been put forward from time to time. In the most favoured hypothesis the membrane limiting the neurosecretory vesicles fuses with cell membrane so that the contents of the vesicle are released directly into the extracellular environment. This process is called as 'exocytosis'.

Release of material from the neurohaemal organs is not the only mode of release. In several cases neurosecretory granules have been observed in the intercellular spaces of the brain. This material parallels the intracellular material moving along the axons. Ultrastructure evidence favours a perikaryal release of material. This material after traversing the brain is then released into the blood. But some workers believe this to be gliosomes and few others are of the opinion that this represents colloids within the dendritic processes of the neurosecretory cells.

Distribution of NSM to the target tissues : Mode of transport of material from the neurohaemal organs to the target tissues is also not definitely known and three different modes of distribution have been suggested. Majority of the workers are of the opinion that the hormones are transported to the target tissues via the haemolymph. Presence of haemocytes in the close vicinity of the neurohaemal organ and other endocrine tissues and material within the haemocytes led some workers to believe that neurosecretion is probably transported to the target tissues via the haemocytes. The transport of hormones via haemocytes would by contrast be a much slower process as it would involve drifting of the haemocytes first to the neurohaemal organs for absorption of hormones and then to the target organs for unloading them. Stained material within the haemocytes is probably the utilized carrier protein or some other material positive to these stains. A third possibility is the supply of material to the target tissues directly by the neurosecretory axons. In this type of neurosecretion, loaded axons innervate the neurohaemal organs and then run out to their target organs very near them.

Nature of the hormones produced by the neurosecretory cells : A number of different hormones are produced by the neurosecretory cells and most important is the brain hormone which controls growth and reproduction. Insect neurosecretory system has striking histological, histochemical and ultrastructural similarities with the hypothalamo-neuro-hypophysial system of vertebrates and hormones produced by the hypothalamic centres are octapeptides associated with inert carrier protein *neurophysine.* It could well be possible that insect neurosecretory hormones would similarly be polypeptides or proteins. But extracts of brain hormone of *Bombyx mori* were reported to be lipid, having properties identical to that of cholesterol. According to another report the brain hormone is a water soluble polypeptide or small molecular protein. Extracts from the brain and corpora cardiaca of cockroaches have more properties of polypeptides and mucoproteins than steroids. But so far, we do not exactly know about the chemical composition of the brain hormone.

Control of the neurosecretory system : Neurosecretory material is synthesized only in the perikaryon of neurosecretory cells, transported down the axons and stored for a shorter or longer time in the swollen axon terminals in the neurohaemal organs. Neurosecretory axons are accompanied by the nerve fibres from the brain. The corpora cardiaca are also in nervous connection with the hypocerebral ganglion. It is not clear whether these nerves carry efferent impulses from the ganglion to the corpora cardiaca or whether they are composed solely of fibres in direct communication with the brain. But due to a spatial separation of the perikaryon and the axon terminals it is quite possible that the control of NSM synthesis may be different from the control of release from the neurohaemal organ. In diapausing pupae of *Hyalophora,* the release of brain hormone to break the diapause is accompanied by the return of electrical activity in the brain and it is possible that the release of hormone is governed by nervous impulses.

In starved females of *Schistocerca,* material accumulates in the corpora cardiaca along the neurosecretory axons and within the perikarya of the neurosecretory cells. Accumulation of NSM is followed by diminution of the neurosecretory cell nuclei. Thus it appears that synthetic activity of the cells becomes reduced when release of neurosecretion is inhibited. Feeding of starved animals results in the immediate depletion of the material from the system and enlargement of nuclei showing that release induces synthesis of NSM.

Functional significance of neurosecretory hormones : It has been demonstrated several times that neurosecretory cells and the corpora cardiaca control a number of physiological processes in insects. Are these varied effects due to the same hormone or a number of different hormones is not definitely known? Of course, in certain cases a differentiation between the hormones can be made, as for example, tanning hormone (bursicon) when injected into *Rhodnius* will not activate the thoracic glands and is consequently different from PG glands stimulating hormone, (thoracotropin), although both are produced by the neurosecretory cells. The growth and reproduction in insects are undoubtedly under the control of neurosecretory hormones, particularly through some aspects of protein synthesis. Activity of the corpus allatum and prothoracic glands and hence synthesis and release of neotinin and ecdysone are also under the control of cerebral hormones. Other hormone-controlled activities are diapause, diuresis, peristalsis, protease synthesis, tanning, wound healing and regeneration. This means to interpret that all vital activities of the body are under neurosecretory control. Factors from the corpora cardiaca accelerate peristalsis, heart beat, thereby increase digestion, transport of metabolites and excretion of waste products. Corpora cardiaca extracts elevate blood sugar level (hyperglycaemia) by activating the enzyme, phosphorylase. Neurosecretory hormones have also been associated with behavioural activities, such as, response of the female towards the male, cocoon formation *etc.*

Carpora cardiaca: Corpora cardiaca are the conventional storage and release organs for the neurosecretion synthesized in the brain and transported down the axons. These are paired bodies usually fused in the middle and the innervated by two pairs of nerves from the brain. The gland is composed of two parts—a nervous part and a glandular part. The nervous part mainly consists of neurosecretory fibres and the glandular part consists of two types of glandular cells—chromophobic and chromophilic cells. Cells of the corpora cardiaca secrete their own neurosecretion. The glands together with hypocerebral ganglion originate as unpaired dorsal evagination of the oesophagus. In the heteropterans neurosecretory fibres bypass the gland and end in the aorta wall. In these insects aorta wall is the storage-and release centre for the material elaborated by the medial neurosecretory cells. In *Calliphora* also neurosecretion is partly stored in the aorta wall and partly in the corpora cardiaca. NSM from the ventral ganglia is stored into a series of neurohaemal organs called *perisympathetic* organs found in the vicinity of the small median ventral nerve.

Corpora allata: These glands were discovered as early as 1899 by Janet and are now known in almost all the chief groups of insects. The simplest and most primitive type is the paired lateral glands attached with paired lateral corpora cardiaca, as in Ephemeroptera and Lepidoptera. Later, fusion of the corpora cardiaca (CC) gave rise to another type where the glands are attached through a nerve to either side of the CC, as in Orthoptera and Dictyoptera. The third type is developed by the fusion of the CA either below the dorsal aorta as in Hemiptera or above it as in Tabanidae. A special type with both CC and CA fused and connected by the fused PG to form a single structure is represented by the ring gland of cyclorrhaphous Diptera. The glands are innervated by a nerve from the CC-nervi corporis allati I (NCA I) and in many species by a second nerve, NCA II from the suboesophageal ganglion. Both the nerves contain axons from neurosecretory and non-neurosecretory neurons.

Corpora allata are spherical ovoid bodies enclosed in a connective tissue and contain three types of cells *viz.*, undifferentiated cells, normal secretory cells and polyploid giant cells. The glandular cells increase considerably during the period of their secretory activity and autoradiographic and histological studies show that increase in the volume is mainly due to the activity of the glands. Ultrastructure studies show that in active glands, the cytoplasm of the cells become more abundant, the quantity of mitochondria and ribosomes increases, and endoplasmic reticulum and numerous granular inclusions appear. The ensheathed membrane gives out processes which penetrate between the secretory cells of the gland and thus forming a 'stroma'. The stroma serves as a pathway through which food and other chemical exchanges between the gland and the haemolymph including diffusion of the hormones take place.

Hormones secreted by the corpora allata : In the larval stages, CA secretes juvenile hormone also called as *neotinin*. Juvenile hormone was first extracted from the abdomen of *Hyalophora cecropia* where it is found in large quantities. A single male abdomen contains about 200 mg of crude yellow oil. When 50 mg of the extract was injected into a pupa, it moulted into a second pupa—an effect similar to the implantation of active CA. In another experiment when the extract was applied locally in the last larval instar of a Hemimetabola or in pupa of Holometabola, the epidermal cells in that region were induced to secrete another larval or pupal cuticle at the next moult whereas the surrounding cells produced a normal adult cuticle. A very high purified juvenile hormone from *Hyalophora* proved difficult to characterize. In the meantime a large number of compounds and extracts were assayed for juvenile harmone activity and yielded interesting information. One such discovery from the faeces of the mealworm, *Tenebrio molitor* is that of the terpene, Farnesol and its oxidation product Farnesal. In *Tenebrio, Rhodnius* and cockroach and several other insects, Farnesol and particularly its methyl ether (Farnesyl methyl ether) and diethyl amine (Farnesyl diethyl amine) are able to produce all the effects of juvenile hormone, as for example, delayed metamorphosis and reversal of adult characters in moulting adults. It seemed conclusive that insect juvenile hormone was Farnesol and its closely related terpenoid compounds. But Farnesol and its derivatives are many times less effective than highly purified extracts from *Hyalophora cecropia*.

Farnesol is present in larger amounts in chilled pupae of *H. cecropia* than in adult males; although the pupae are

Substance	Juvenile hormone activity (*Galleria* units/g)
Farnesol	16
Farnesal	32
Farnesyl methyl ether	1000—1500
Farnesyl diethylamine	1000—1500
Crude *H. cecropia* extract	1000
Concentrated *H. cecropia* extract	300,000,000

completely devoid of juvenile hormone, the adult males contain enormous amounts of juvenile hormone. Secondly allatectomy in *H. cecropia* completely prevents the appearance of juvenile hormone in adults but the contents of Farnesol are unaltered. Thirdly, Farnesol and their derivatives mimic the effects of juvenile hormone in some insects but not in others. The results completely go against the idea that Farnesol and its derivatives are actually juvenile hormones. Although these compounds mimic the effects of JH, these are not themselves JH.

In 1967 Roller and his colleagues at Wisconsin discovered the structure of JH as 'methyl 10—exopy—7 ethyl—3, 11-dimethyl 1—2, 6 tridecadienoate'. Another important discovery is that by Law and his coworkers at Harvard. Law *et al.*, obtained a potent JH mimic from commercial Farnesol by a relatively simple method. Law's mixture is said to be 1000 times more active than the crude *H. cecropia* oil. The nature of the hormone in adult insects is still a big problem before the endocrinologists.

Control of CA activity : Most thoroughly studied and least understood is the mechanism of control of CA activity, although attempts to study the control of these glands are in progress for the last 20 years. Several possible mechanisms have been proposed. (1) The glands may be directly stimulated by the neurosecretory cells from the brain. In *Locusta, Schistocerca, Tenebrio* it has been demonstrated that severing the NCA I between the CC and CA prevents glandular activity. Ultrastructure and light microscope studies in a number of insects have shown the presence of NSM in the NCA I and CA during periods of activity and its absence during periods of inactivity. Therefore, it appears that in some cases direct innervation of CA by the neurosecretory cells facilitates the transfer of hormone which acts as the activation hormone and stimulates the glandular activity of CA. Through a series of experiments it was demonstrated that lateral rather than medial neurosecretory cells are responsible for CA activity. The second possibility is that the neurosecretory substances indirectly affect CA activity. In *Calliphora* neurosecretory cells are required for CA activity but there is no innervation of neurosecretory axons in the glands. (2) In indirect control hypothesis, the neurosecretory cells which control haemolymph protein concentrations, control CA activity by regulating haemolymph protein levels. This idea is based on the fact that denervated glands in the sugar-fed flies remained small while became larger in meat-fed flies. (3) A third mechanism of CA control is derived from the studies on *Leucophaea* and *Oncopeltus*. In these insects cutting the NCA I results in activation of the inactive glands. The source of the inhibition seems to be certain neurons in the brain lateral to the neurosecretory cells. Since both in *Leucophaea* and *Oncopeltus* medial neurosecretory cells are needed for CA activity, it appears that in these insects stimulation is under neurosecretory control and inhibition is under nervous control.

A decreasing activity of corpora allata during successive larval instars resulting in the virtual cessation of activity in the last instar is at present explained. The suggestion that increase in growth of the gland during successive moults is insufficient to maintain adequate surface area for secretion into the increasing volume of haemolymph in successive instars, does not seem to be valid. The denervation of CA in the last instar results in the secretion of sufficient amount of hormones and results in supernumerary moults. It seems more likely that trophic relationship between neurosecretory system, CA and PG provides a basis for determining the number of instars through which an insect have to pass before reaching adult stage. A variety of other factors such as feeding, temperature, photoperiod, copulation, oviposition and injury influence the activity of CA.

Function of CA hormone(s) : CA hormones are directly or indirectly involved in a large number of physiological activities. Most important direct effects are growth and reproduction. Implantation of active CA at the beginning of the last larval instar partially or completely prevents metamorphosis and results into adultoid forms or supernumerary larval instars. The removal of CA from early larva results in miniature adult. These and a number of other experiments suggest that CA hormones are responsible for the inhibition of metamorphosis. The CA hormone(s) is therefore sometimes also called as 'inhibitory or status quo' hormone. A number of experiments show that this hormone is necessary for the development of eggs in females and functioning of the accessory glands in the males. Effects of allatectomy in males is less conspicuous. In several cases allatectomy suppresses egg maturation while in several others it has no effect. The possible explanation for such an anomalous situation is that in the absence of CA hormone, neurosecretory cells take up the function of secretion of gonadotrophis. Other CA controlled activities are polymorphism, regeneration, metabolism of fat, water balance, colour changes, imaginal diapause, pheromone production *etc.*

Use of juvenile hormone and their mimics in pest control: During normal insect development juvenile hormone is necessary at precisely determined periods. Its presence during the last larval instar can prevent metamorphosis and may cause death of the insect without becoming adult. In particular, JH must be absent from the eggs otherwise the embryonic development is abnormal and larvae do not hatch. Thus JH and its mimics if administered at wrong times in insect life histories could be used as insecticides. Such compounds would have tremendous advantage over insecticides presently used, since the insects would be unlikely to develop resistance to substances which play a natural role in their own lives. Some juvenile hormone mimics are more effective in some species than in others so that the development of specific insecticide could also be possible, in contrast to compounds like DDT which kill destructive and beneficial insects alike.

The concept of juvenile hormone and their mimics as insecticide was developed in a startling way when Dr. Karl Slama of Prague went to Harvard to collaborate with Professor Caroll Williams. Slama took a stock of the bug *Pyrrhocoris apterus* with him but he found that in Harvard the bugs would not develop into normal adult. Instead, at the end of 5th larval instar all insects moulted into a supernumerary 6th larval instar or into adultoids which preserved many larval characters. A few even grew and moulted into 7th instar larvae. These effects are similar to those of implanting corpora allata into last instar larvae. Thus it appears that *Pyrrhocoris* were being unintentionally exposed to a source of juvenile hormone activity.

A comparison between the culture conditions in Harvard and Prague eventually revealed that paper towels used in rearing jars were the source of the substance. Later on, it was found that all papers, including news papers of American origin had the same effect upon *Pyrrhocoris* but those of European and Japanese manufacture had no effect. Majority of wood pulp used for paper manufacture in the United States is the *Balsam fir* (*Abies balsamea*) and extracts from this tree proved to contain high juvenile hormone activity when assayed on *Pyrrhocoris*. The substance was called as 'paper factor' which chemically show resemblance to JH. The paper factor proved to be ineffective on *H. cecropia* and even *Rhodnius prolixus* (bugs belonging to the same order). On *Dysdercus* it is as effective as on *Pyrrhocoris*. This raises the possibility of using such chemicals as specific insect pesticides. JH and their analogues have already shown interesting results against *Tribolium* infestation. Recently a technique has been developed which could provide specific control of insect pests in the field. In *Pyrrhocoris* a dihydrochloride of methyl farnesoate (DMF) seriously disturb embryonic development when applied to the eggs, and less than 1 μ g applied to a mature female causes her to lay inviable eggs for the rest of her life. The males of *Pyrrhocoris* will tolerate up to 1000 μ g of DMF when applied topically but will pass sufficient DMF to females during mating to sterilize the latter for rest of their life. Males with 1000 μg can pass 1-5 μ g to females within 4 hrs. With much more potent compounds even 10 μg/male will be sufficient. This method has the great advantage that females once mated with a treated male will be sterile for life no matter if she subsequently mates with any number of untreated normal males. Hormonal insecticides, with their advantages to specific control over individual pests are likely to prove most effective insecticides in terms of increased production and reduced wastage of food, coupled with their ability not to induce immunity in pests.

Prothoracic glands: As early as 1922 it was known that humoral factors are responsible for the control of moulting and that these factors are located in the thorax. First experimental proof about the importance of prothoracic glands was provided by a Japanese worker, Fukuda in 1940 working with silkworm. Since then prothoracic glands or their homologues have been described in several insects. Although highly variable in shape, the glands have certain features common in all groups where these occur. These are paired glandular structures located in the ventro-lateral areas of the prothorax and are sometimes partially fused in the middle. These are abundantly supplied with tracheae and are fixed in the body cavity with the help of muscle fibres and the nerves from the prothoracic or suboesophageal ganglia. The glands are almost transparent in the living insects. These gradually increase in size and reach their maximum in the last larval instar. In the adult insect these degenerate within 2-10 days of the imaginal moult. The glands during each cycle reach their maximum size about 24 hrs after cessation of feeding which is the critical period for many insects.

Histologically the prothoracic glands are generally composed of two rows of glandular cells which show cyclical activity very similar to those of the cells of the corpora allata. Their cytoplasm contains basophilic and lipoidal granules. The glands develop as an epidermal invagination of the lateral portion of the maxillary segment and extend into prothorax.

Other homologues of the prothoracic glands are ventral glands found in the hind ventral region of the head of Phasmida, Odonata and Isoptera. Like prothoracic glands these also degenerate immediately after the imaginal moult. In the workers and the soldiers of termites, these are active during the whole life. Pericardial and peritracheal glands, although developed differently, are structurally and functionally identical with the prothoracic and ventral glands. A special endocrine organ which combines the source of three metamorphic hormones is found in cyclorrhaphous Diptera and is referred to as 'Weismann's ring'. It consists of (i) corpus allatum, (ii) peritracheal glands, (iii) corpus cardiacum and (iv) hypocerebral ganglion.

Hormones secreted by the prothoracic glands: In 1954 Butenandt and Karlson for the first time extracted a steroid hormone, *ecdysone* in crystalline form from *Bombyx*. About 500 kg of silkworm pupae yielded only 25 mg of ecdysone. The crystalline ecdysone induces pupation in ligatured blowfly larvae when as little as 0.0075 μg is injected and this quantity of hormone is known as the "Calliphora unit (CU)." Ecdysone probably has at least three factors. These are α-ecdysone ($C_{27}H_{44}O_6$), β-ecdysone ($C_{27}H_{44}O_7$), and ecdysterone which seems to be more potent than the α or β forms. Ecdysone like material with marked biological activity has now been isolated from leaves and roots of ferns (*Podocarpus* and *Achyranthis*). Ecdysone has the same carbon skeleton as cholesterol and when radioactive cholesterol is administered to *Calliphora* larvae, a great deal of the radioactivity is incorporated into the PG. Cholesterol, therefore, appears to be precursor of this hormone in insects. The existence of two or three (may be more) ecdysones in individual species is of considerable interest. But it is not as yet known whether these forms have specific effects upon different aspects of metabolism and development, or these are precursors or breakdown products of a single steroid hormone.

A large number of ecdysone mimics have been synthesized from the plants and their possibility to use in the control of insect pests are not as encouraging as those of juvenile hormone because ecdysone mimics are active only when injected. Topical application proved a failure. Even if used as a bait it was observed that ecdysone was not absorbed into the alimentary canal.

Control of prothoracic glands: The prothoracic glands are innervated from the suboesophageal and thoracic ganglia. The significance of such an innervation is not fully known. It is possible that these nerves are involved in the destruction of the glands during the final moult. The glands are activated by the thoracotrophic hormone from the neurosecretory cells and produce and release their own hormones into the blood. Both medial and lateral groups of neurosecretory cells are necessary for PG activation.

Prothoracic glands may also be activated by juvenile hormone from CA but how this mechanism works is not well understood. This is substantiated by the fact that in those insects in which development is delayed or accelerated, removal or implantation of CA degenerates the thoracic glands. Further support to this view is derived from the experiment that injection of the juvenile hormone enhances RNA synthesis in the thoracic glands of the larvae and pupae.

Some workers are of opinion that prothoracic glands may be activated to produce moulting hormone even in the absence of any 'activation hormone'. Diapause in *H. cecropia* pupae may be broken by the implantation of active prothoracic glands or by the injection of such small amount of ecdysone into pupae with inactive prothoracic glands which may not be sufficient to initiate the moulting process in specimens with their prothoracic glands removed.

Functional significance of ecdysone : Ecdysone is also called as moulting and metamorphosis hormone. Most important function of ecdysone is the initiation of moulting process in insects. There is sufficient evidence that prothoracic gland hormone directly affects the breaking of larval and pupal diapause. Views have been expressed that moulting hormone interferes with principal enzyme system and regulates normal metabolism and growth, as for example cytochrome oxidase system. It is also now confirmed that ecdysone influences 'puffing' patterns of giant chromosomes in the salivary gland cells in *Drosophila* and *Chironomous*. The other effects of ecdysone are regeneration, colour changes and inhibition of the function of CA.

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PHEROMONES

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IN addition to sound and visual mode of communication, various organisms also use chemical signals for transmitting information among the individuals belonging to same species. In insects, this phenomenon of chemical communication has been known since 1837. Certain types of chemicals released by insects have been recently given the name of pheromones by Karlson and these are defined as, "Chemical substances which are secreted to the outside by an individual and received by the second individual of the same species, in which they release a specific reaction." Thus the pheromones could be termed as chemicals used for intraspecific communication. The pheromones are useful to the species *e.g.*, in sexual activity such as location of opposite sex and indeed it has been claimed that sex pheromones of insects are among the most potent and physiologically active substances known today. Other types of pheromones released are *alarm* pheromones and *recruitment* pheromones by social insects. These are utilized for collective defence or increased foraging activity. Like other methods of communications pheromonal communication also requires (a) a generator or transmitter of signal and (b) a receptor or receiver for the signal to elicite the required response.

In the following pages are given the chemical structures of the pheromones and the insects which produce these. It is apparent that a number of relatively simple compounds of low molecular weight (80-300) can perform the function of a pheromone. It is noteworthy that majority of these are derivatives of fatty acids or terpenes which not only have a low molecular weight but are also of lower boiling point and show a high degree of vaporisation. The sex pheromones are usually of two types (a) pheromones, released by the females to entice the males and (b) those released by males to attract females.

Pheromone production and release

Pheromones are generally produced by the external glands. In almond moth, *Ephestia cautella* this is secreted by the

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* Since 17.1.1977.

eversible glands in the last 3 abdominal segments of the females. In males, these may be produced either in the last abdominal segment, as in hair pencil in the butterflies of genera *Danaus* and *Lycoria*, or as androconia on the wings in *Galleria mellonella*. The production of alarm and recruiting pheromones is either by mandibular, and or sting glands. The pheromone production appears to be under the control of hormones secreted by corpora allata as has been elegantly proved in case of cockroaches like *Blaberus, Leucophaea* and *Periplaneta*. Despite the extensive knowledge of the existence of the sex pheromones, there is very little information available about the biochemical route of their synthesis inside the body. Logically the biochemical pathway must be related to synthesis of fatty acids or terpenoids. This makes the whole area of biochemical synthesis of pheromones a fascinating subject for investigation. Recently it has been shown that certain butterflies are able to convert mevalonic acid to monoterpenes. Since these monoterpenes also occur in plants, it is likely that in some insects at least, potential pheromones are collected from the plants and then concentrated and used. Release of pheromones by the insect is dependent on a number of ecological factors *e.g.*, weather and the time of the day. *Protoparce sexta*, females produce sex pheromones between midnight and 1 A.M. and *Trichoplusia ni* from 1 A.M. to 5 A.M. In other insects like *Antheraea polyphemus*, the female release the sex pheromone only if the atmosphere contains certain percentage of trans-2-hexanol. Trans-2-hexanol is produced by the leaf of the host trees and which triggers the release of this pheromone by the females.

Transmission and perception

Sex attractant released in the atmosphere is able to attract the members of the opposite sex from quite a long distance. It has been shown that the males of *Diabrotica* spp. in the field are lured from a distance of 40-50 feet by virgin females or a methanol extract of their abdomens. The amount required to attract the opposite sex is very small. Bombykol, a sex attractant produced by the females of *Bómbyx mori* works at a concentration of 10^{-12} μg. Female of *Porthetria dispar* is able to attract males at a concentration of 10^{-10} μg. A number of theoretical studies have been made by which it is possible to calculate the minimum concentration of pheromone necessary to trigger behavioural response. This minimal concentration of the pheromone is known as *Behavioural Threshold Concentration* (BTC). If BTC and the amount of pheromone are known it is possible to calculate (a) useful distance of communication, (b) time required for signal transmission and (c) duration of signal necessary to be effective. Thus if Q is the emission rate and K is behavioural threshold concentration or BTC then Q/K value 1 cm^3/second will have the maximum transmission radius of 0.6 cm, Q/K value 10^6 cm^3/second will have a perception radius of 6 cm. Since most of the sex attractants have Q/K value of 10^{10}—10^{12} cm^3/second this would suggest that their minimum perception range would be at least 1 metre and above.

Once the pheromones are released in the atmosphere these elicit a typical behaviour in the members of the same species *e.g.,* in case of *Bombyx mori* the males of the species show sexual excitement characterized by circus movement, rapid wing vibration and bending of abdominal extremity towards the head. This type of behaviour is characteristic of insects exposed to sex pheromones.

Pheromones could be classified into two types (a) *releasers* and (b) *primers,* depending upon the type of the behaviour these manifest in the recipient individual. Releasers are those pheromones which immediately elicit the response from the recipient individuals. Under this category come sex pheromones, alarm and trailing pheromones *etc.* Primer pheromones are those in which the behavioural response is slow to manifest, *e.g.,* queen bee substance, (9-keto-2-decenoic acid) which prevents development of gonads in workers. Other types of primers are some of the sex pheromones. In *Danaus* sp. females release a pheromone to attract the males which show a characteristic courtship behaviour but the female does not copulate with the male unless and until it releases a primer sex pheromone which makes the female receptive to sexual advances by the males.

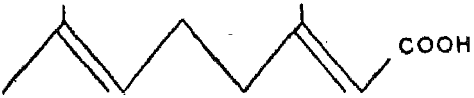
The manner in which a moth or any insect finds a male has inspired biologists and biochemists to propound a number of theories about the mechanism of perception. The earliest theory regarding this was propounded as early as 1894 by Riley to explain the phenomenon in which he observed a male moth following the trail of a female one and a half mile away and he described this due to telepathy. Since then a number of theories have been propounded, *e.g.,* the theory of Dyson which tried to correlate the odour with molecular vibration which he tried to relate with Raman spectra. Similarly Duane and Tylor tried to explain the atraction due to infra red spectrum. The latest of these physical theories is that by Callahan who suggested an electromagnetic radiation force of attraction between the sexes as measured by the infra red spectra. The theory which is currently in vogue is that by Amoore which he explains on the basis of *Lock* and *Key* model. Indeed recent experiments carried by Riddiford suggests that the antennae contain a specific protein which actively binds with the sex pheromone, and this protein is not present in the antennae of the sex which releases to the sex pheromone.

The highly specific nature of the sex pheromones can prove an asset for surveying and control of insects in near future.

RECRUITING PHEROMONES

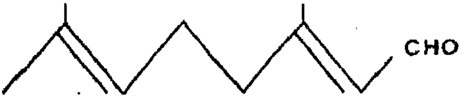
HONEY BEE

a.



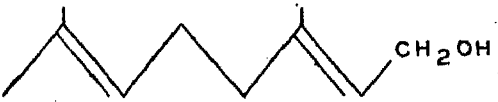
Geranoic acid

b.



Geranial

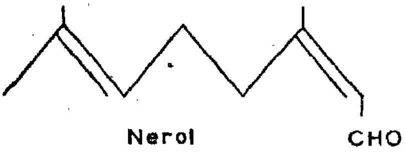
c.



Geraniol

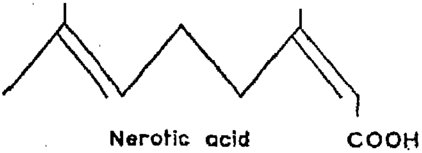
SOLITARY BEE

a.



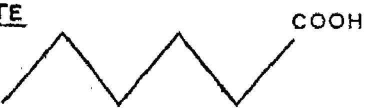
Nerol

b.



Nerotic acid

TERMITE



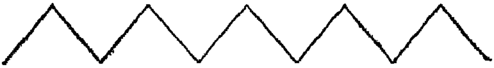
Hexanoic acid

The insects by responding to a lure baited trap are caught and thereby signal the presence of species in the area. Such

ALARMING PHEROMONES

ANTS

a.



Undecane

b. Tridecane

c. Pentadecane

HONEY BEES

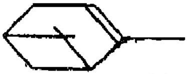
a. Iso – amyl acetate

b. Analogs of methyl heptane - 4 - one

c. Trans hexanal

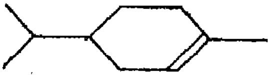
WHITE ANTS

a.



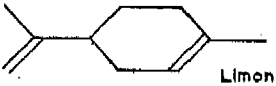
Pinene

b.



Terpenolene

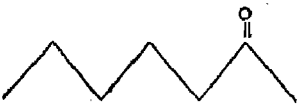
c.



Limonene

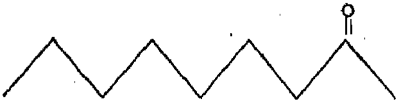
HYMENOPTEROUS INSECTS

a.



Heptane – 2 – one

b.

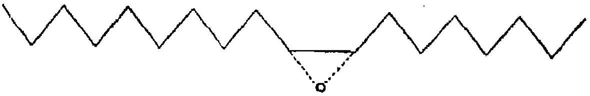


Nonan – 2 – one

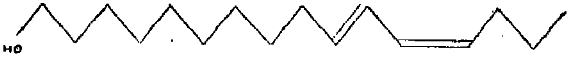
traps have been extensively used for surveying the populations of Gypsy moth *Porthetria*, and pink bollworn *Pectinophora gossypiella*.

SEX PHEROMONES

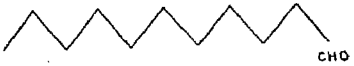
Porthetria dispar



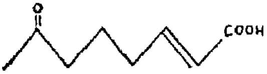
Bombyx mori



Galleria mellonella (male)



HONEY BEES



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20

ROLE OF BEHAVIOUR IN HOST PLANT SELECTION BY INSECTS

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INSECTS show marked differences in the degree to which they infest different plants in a locality. Some insects attack most of the species of plants available whereas others confine themselves to a few or only one of them. Under identical ecological, phenological and geographical conditions, the above differences in the infestation of plants by different insects are determined by physiological factors. These factors involve an interaction of different responses of insects to plants and various characteristics of the plants. These responses may be arranged under the following six categories according to the stages in which insects may be regarded to establish their populations on plants: (1) *orientation*, resulting in the arrival of insects on a plant, (2) *feeding* of the insects arriving on the plant, resulting in food intake, (3) *metabolism* of the ingested food, determining *nutrition* of the insects, (4) *growth* of the insects, (5) *survival* and *egg-production* in the adult stage of the insects and (6) *oviposition* (Saxena, 1969).

Of the above six types of responses, the first two and the last are behavioural and are involved in the initial selection of a plant by an insect. Nutrition, growth and egg-production are involved only in subsequent stages of the insect's establishment. Orientational responses of an insect to plants may be positively directed, resulting in its attraction to arrest at the plants or negatively directed resulting in the insect's repulsion from or avoidance of the plants. The arrival of an insect on a plant, whether by accident or as a result of its orientational response, may or may not be followed by feeding. If feeding occurs, the subsequent stages of the establishment would follow. Differences in the orientational and feeding responses of different insects to different plants would thus be responsible for the selection of some and not of other plants. The above responses of insects are determined by (i) various sensory stimuli, emanating from the plants and (ii) factors governing the insect's responses to the above sensory stimuli.

Role of Sensory Stimuli

Several workers have studied the role of different sensory stimuli from plants in determining orientational and feeding responses of different plant-infesting insects and the relevant literature has been included in the reviews of Thorsteinson (1960), Beck (1965) and Schoonhoven (1968). The stimuli from plants may be physical or chemical. The most important of the physical stimuli are visual and mechanical. Visual stimuli may be provided by colour and shape of plants. Mechanical stimuli from plants include their texture, hardness, hairiness *etc*. Chemical stimuli from plants may be of three types: (1) water as vapour (humidity) or liquid, (2) odour and (3) taste or other contact chemical stimuli.

The above sensory stimuli from different plants may differ in following respects: (1) distance of their perception by insects, (2) behavioural responses elicited by them from different insects and (3) their presence or absence in different plants.

With reference to distance of perception, some stimuli, *e.g.*, visual, water vapour, odour, are perceived at a distance from their source. Others, *e.g.*, mechanical, water (liquid), taste, are perceived only by contact with the source.

Regarding the types of behavioural responses elicited by the above stimuli, the orientational responses of insects, may be positively directed, resulting in attraction to arrest at the stimulus source or negatively directed resulting in avoidance of the plants concerned. Similarly, feeding responses of the insects may be positive or negative, *i.e.*, inhibited by various stimuli.

Considering the presence or absence of these stimuli, some may be common to most, if not to all plants available in a locality while other stimuli may be confined to one or a few of these plants. The sensory stimuli which elicit positive responses of an insect but occur in the preferred as well as non-preferred plants, may be regarded as *non-specific* since these cannot provide *cues* to insects for discrimination among the selection of different plants. On the other hand, the sensory stimuli which elicit orientational and feeding responses of an insect but are restricted to certain plants serve as *specific stimuli* since they provide a cue to the insect for discrimination among and selection of some of the available plants. If these specific stimuli elicit positive responses from an insect, their presence would render the plants acceptable. On the other hand, if the specific stimuli cause negative responses, their presence in plants would render them unacceptable and the plants lacking such specific stimuli would be preferred and selected by the insects.

Visual stimuli from plants include their shape and colour. There are a few reports indicating that certain phytophagous insects *e.g.*, *Papilio demoleus* (Vaidya, 1969) can perceive different shapes. But, there is no evidence to indicate that differences in the shape of preferred and non-preferred plants can bring about their selection or rejection by an insect.

Colour of plants has also been reported to determine orientational and feeding responses of a number of insects (Kennedy, Booth and Kershaw, 1961; Moericke, 1969). As the leaves of many preferred and non-preferred plants are almost of the same colour, doubts are sometimes raised that plant colour would be a non-specific stimulus which cannot provide a strong cue to insects for discrimination among plants. However, Moericke (1969) considers the role of three parameters of colour: (i) the *hue* referring to wave length in the spectrum, (ii) the *tint* referring to the amount of white added to the hue, changing it from saturated to unsaturated colour and (iii) the *intensity* which can be changed and diminished by adding black to the hue. According to him, colour of leaves of different plants does not differ much in hue which ranges generally around yellow green (about 550 mμ) but differs in tint and intensity. Moericke (1969) considers that phytophagous insects may show preference for a specific tint or a specific intensity of colour of their preferred plant. For example, the mealy plum aphid *Hyalopterus pruni* after leaving the plum and when looking for its summer host, *Phragmitis communis,* alights on the latter in response to unsaturated green (grey-green) colour of the leaves rather than on beet plants which have a saturated green colour.

Although further evidence is needed to understand the role of above parameters of colour in determining selection of plants by insects, it appears possible that these parameters may serve as limited specific stimuli.

Water, present in plants, may serve as *distance* (vapour) as well as *contact* (liquid) stimuli which may determine orientation and ingestion in a number of insects. For example, the red cotton bug *Dysdercus koenigii* when desiccated is attracted to preferred as well as non-preferred plants in response to the water stimulus in the form of vapour (humidity) and liquid sap (Saxena, 1964, 1969). In general, most plants appear to provide more or similar water-stimuli which are thus non-specific and cannot provide useful cues for discrimination among the preferred and non-preferred plants.

Various *mechanical* characters (hardness, hairiness, *etc.*), are also known to differ among plants and to play some role in feeding and oviposition by insects. These characters serve mainly as barriers or permissive factors (Thorsteinson, 1960) rather than as sensory stimuli influencing insects' behaviour. Although these factors are more specific than visual or water stimuli, there are many plants which are identical in the above mechanical characters and still differ in their selection or rejection by different insects.

Several chemical constituents of plants serving as *olfactory* and *gustatory* stimuli have been reported to differ qualitatively and quantatively in different plants and the relevant literature is included in the reviews of Thorsteinson (1960), Beck (1965) and Schoonhoven (1968). These chemicals may be nutrients, *e.g.*, sugars, amino acids *etc.*, or non-nutritive contituents, *e.g.*, glycosides, alkaloids, terpenoids *etc.* Such stimuli are specific and are regerded to be most important determining selection or rejection of plants by insects (Dethier, 1953; Kennedy and and Booth, 1951; Fraenkel, 1959; Thorsteinson, 1960; Beck, 1965; Schoonhoven, 1968), as illustrated in a few examples below :

A number of mustard oil glycosides, *e.g.*, sinigrin, sinalbin, are present in cruciferous plants and are regarded as specific chemical stimuli responsible for attraction and feeding of the larvae of *Plutella maculipennis* on the above plants (Thorsteinson, 1960). Specificity of *Bombyx mori* larvae to mulberry leaves has been shown to depend on the olfactory stimuli (citral, terpinyl acetate, linalyl acetate, linalol, β-γ-hexanol and α-β-hexanol and gustatory stimuli (β-sitosterol, isoquercitrin, cellulose, sucrose, inositol, ascorbic acid, phosphate and silicate) (Hamamura, 1959; Hamamura *et al.*, 1962; Horie, 1962; Ito, 1961; Nayar and Fraenkel, 1962). Restriction of the feeding of the Mexican bean beetle *Epilachna varivestis* to plants of the genus *Phaseolus* appears to be due to the presence of the cyanogenetic glycosides, phaseolunatin and lotaustrin which serve as specific gusatory stimuli at low concentrations (Nayar and Fraenkel, 1963). Specific olfactory and gustatory stimuli which determine attraction and feeding of the cotton boll weevil *Anthonomus grandis* on cotton plants have been characterized by the presence of attractants consisting of a mixture of several chemical compounds, *viz.*, pinene, β-limonene, β-caryophyllene, β-bisabilol, caryophyllene oxide and two unidentified ses queterpenoids (Minyard *et al.*, 1969), and *feeding stimulant* which is a mixture of imperfectly identified polar constituents, including carbohydrates (Hedin *et al.*, 1966; Struck *et al.*, 1968).

Presence of certain chemicals in plants may cause their rejection by some insects. For example, the colorado potato beetle *Leptinotarsa decemlineata* shows a strong feeding preference for potato and a few other solanaceous plants but much less or none to certain other solanaceous plants, e.g., *Solanum nigrum, S. luteum, etc.*, (Hsiao and Fraenkel, 1968; Bongers, 1970). Such a low or lack of feeding response of the insect to the above plants believed to be due to the presence of certain alkaloids *e.g.*, tomatine, demissin, leptine *etc.*, which serve as repellents or feeding inhibitors (Schreiber, 1958; Stuckow and Low, 1961).

The above mentioned olfactory and gustatory stimuli which appear to be most important in providing specific cues to insects for discrimination among plants, are perceived above the threshold concentration over fairly short distances. In undisturbed air, caterpillars of *Cingilia catenaria* perceive the odour from the leaves of their food plant up to a distance of about 5 mm only (Dethier, 1941). Larvae of *Bombyx mori* (Torii and Morii, 1948) and *Hyponomeuta euonymellus* (Kalkowski, 1966) can be attracted by the odour of their food plant up to 3 cm. Certain grasshoppers, *e.g.*, *Melanoplus*, can be attracted by the odour of food up to 40 cm (Slifer, 1955). Wind borne odours can also be perceived by insects and elicit their upwind movement, *e.g.*, in *Schistocerca gregaria* (Haskel *et. al.*, 1962) and *Leptinotarsa decemlineata* (de Wilde *et. al.*, 1964). But the distances up to which an odour may elicit behavioural response from insects under different wind velocities have not yet been determined for plant odours. Although these distances are expected to be greater than those in still air, it is not likely that wind borne odours can be operative over long distances. The gustatory stimuli, however can be perceived only when insect is in contact with the plant.

In view of the above, it is evident that insects can exercise discrimination and select a plant only when they come within the distance of operation of the specific olfactory/gustatory stimuli emanating from the plant. The mechanisms for the arrival of insects within the above perception zone are not yet fully understood. There is a possibility that insects perform random movements which may, at one time or another, bring them, within perception range of specific stimuli from various plants. Another possibility is that certain non-specific stimuli may be perceived over longer distances and may bring the insects within the perception range of the specific stimuli. Of the different non-specific stimuli, visual ones appear to operate over the longest distances. Such visual stimuli, though common to preferred and non-preferred plants, can attract insects and bring them within zone of perception of specific olfactory and gustatory stimuli from these plants for final discrimination and selection or rejection. However, the role of random movements and non-specific stimuli in the selection of plants by insects needs further study.

Factors governing behavioural responses of insects to sensory stimuli

A number of workers have reported that orientational and feeding responses of insects to same sensory stimulus may vary at different times, *e.g.*, certain aphids show attraction to blue-violet colour during "take off" flights and to yellow-green colour during "alighting" flights (Kennedy and Stroyan, 1959). Similarly, behavioural responses of insects to other stimuli, including specific olfactory and gustatory stimuli from plants may also vary from time to time. Various factors responsible for such changes in the responses of insects may be arranged under two main categories (i) physiological condition of insects and (ii) competition among different sensory stimuli (Saxena, 1967).

The physiological conditions of insects which may determine and modify their orientational and feeding responses are: life-history stage, (b) stage of moulting process, (c) nutritional state or starvation, (d) desiccation, (e) reproductive state *etc.* (Saxena, 1967).

The red cotton bug *Dysdercus koenigii* shows attraction and feeding response to its perferred food source (cotton seed) only after a certain time interval following the preceding ecdysis. In the larval stage, the insect ceases to show the above responses after it has reached a critical stage of moulting. Even during the moulting stage when the insect shows attraction and feeding response to cotton seeds or their specific olfactory and gustatory stimulants, these responses are elicited from water-satiated individuals but not from desiccated ones (Saxena, 1962, 1964, 1967, 1969). On the other hand, desiccated insects show attraction to high humidity and ingest sap from the leaves of preferred as well as non-preferred plants in response to water stimuli. When water-satiated, the same insects avoid high humidity and water-rich leaves, move to dry zone and show attraction and feeding response to the olfactory and gustatory stimuli from cotton seeds. In the adult stage, immature females, but not gravid females, show the above responses to specific stimuli from cotton seeds.

As regards the role of *competing stimuli,* when specific olfactory and gustatory stimuli are placed in a high humidity zone, water-satiated and starved insects would, on the one hand, be attracted to the specific stimuli and, on the other, repelled by high humidity. If their starvation is not high, repulsion from high humidity is stronger than attraction by the specific stimuli, preventing their selection of the food source by the insect (Saxena, 1967).

The above facts suggest that changes in physiological condition of insects and combination of different sensory stimuli perceived by them during their movements would play important role in determining their responses to specific sensory stimuli, when they arrive within their perception range, and subsequent success or failure in the selection of preferred plants.

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21

INSECT NUTRITION IN ECONOMIC ENTOMOLOGY

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INSECTS are the example of a successful class of animals which, owing to their adaptative evolution survived the onslaught of the changing environment through geological era. So severe were the changes that even the kingdom of the glorified reptiles which once ruled this planet, withered away with an astonishingly rapidity. The insects because of their ability to seek food of different types, to modify their organs to suit different ecological requirements, to make use of microclimate, to their small size and to develop true wings to disperse themselves to new regions, could have the largest number of species in the animal kingdom.

Insects inflict damage to human health and economy in their efforts to obtain food. They associate with our crops with the main aim to secure nutrition for themselves or for their progeny. The extent and nature of damage is determined by the type of relationship between insects and their hosts. A knowledge of their nutritional requirements is therefore of great importance to an economic entomologist. Nutrition also influences the phenomenon of host selection. It investigates into the process by which insects feed and utilize food substances. It therefore concerns an important sphere of insect activity which directly or indirectly determines their economic status. A thorough and deep knowledge of nutrition in its wide sense can explain how one crop plant or its varieties are differentially damaged. In this way insect-plant relationship offers the bases for the susceptibility or resistance of different plants to insects and can help in identifying plant varieties capable of resisting insect attack.

The significance of insect's ability to develop on different food plants has been realised by the earlier entomologists. The literature is full of such valuable data where a pest, though serious in a particular crop, has a number of alternate host plants. This can be regarded as an earlier attempt to see the effect of different foods on insect development, growth, survival and fecundity. Uvarov (1929) and Brues (1946) have reviewed the earlier work on the subject. Owing to the lack of knowledge about the effect of different nutrients on insects, it was not possible to interpret these data in physiological terms. These types of investigations remained within the purview of biological notes and lacked in proper interpretations because of the unknown and complex chemical nature of the plant and animal tissues.

The work carried out on insect nutrition by Fraenkel and his associates during forties in Imperial College, London, made it possible to raise insects on diets of known chemical composition. This period was also marked by the tremendous advancements made in the field of biochemistry and isolation and synthesis of several compounds present in living tissues. Because of the availability of various vitamins of B complex in pure form, synthetic diets for several insects could be evolved. The vitamins like pantothenic acid, nicotinic acid and biotin were identified or synthesized only in 1940. For a very long time the test insects for nutritional studies were stored product pests which feed on dry diets. Now, it is possible to grow several species of phytophagous insects on chemically defined diets. To mention some are: *Chilo zonellus, Pectinophora, Anthonomus grandis,* locusts, aphids, silkworm *etc.* Limited success has been achieved in case of parasites and predators. Reviews by the several authors (Trager, 1953; Levinson, 1955; Lipke and Fraenkel, 1956; Friend, 1958; Legay, 1958 ; Auclair, 1963 ; Beck, 1965 ; Waldbauer, 1968) are available and should be consulted for detailed information.

Nutritional studies and resistance to insects in plants: One of the methods of insect control is to utilize plant varieties resistant to insect. Painter published a book in 1951 entitled *Insect resistance in crop plants* where he has discussed this phenomenon of resistance in several crop plant varieties. He had little information at that time on the causes of resistance. If this type of insect control is to be exploited successfully we must have a thorough knowledge of the factors responsible for resistance. In tropical countries like India each crop suffers from the attack of several species of insects and a caution is necessary not to declare a variety resistant unless true nature of resistance is studied. The physiological and biochemical bases of host plant relationship therefore have to be identified. Plants have several unidentified factors which govern the insect establishment. These could be nutrients or secondary plant substances like alkaloids, essential oils acting as attractants or repellents.

Isolation and identification of these classes of chemicals in plants can help the plant breeders to select proper plant types for evolving resistant varieties. The nutritional status of different biotypes of the same species of the pest must also be known to decide whether a variety will be able to maintain its resistance in a new locality or not.

Nutrition of beneficial insects: There is a great scope of utilizing nutritional data for proper exploitation of beneficial insects like honey bee, lac insect, silkworm, parasites and predators. The significance of nutritional data of these forms is self evident. It is of the same importance to these insects as animal nutrition to livestocks. Nutrition directly influences quality and quantity of the product produced by these insects. Honey, lac or silk-yields can be greatly increased and their quality improved by proper dietary management. Efficiency of a parasite or a predator can be enhanced by providing proper nutrition.

Japan has made phenomenal progress in the nutrition of silkworm. It is now possible to grow silkworm in an artificial diet and the time is not far away when silk industry will no longer depend upon the availability of mulberry plant. During winter months mulberry leaves are not available and insects undergo diapause. Methods are now known to break the diapause. Silkworm rearing can be a continuous process once an artificial diet is perfected.

Unfortunately no attention has been paid to lac insect nutrition with the result the lac production in the country is becoming costlier and scanty. If this industry has to compete with several synthetic lac substitutes' scientific principles must be applied to lac production. It is well known that quantity and quality of lac are dependent upon the type of trees on which lac insects feed. What makes one species of host more suitable than other? What are the factors which affect the metabolism leading to lac secretion? These and other related questions though vital, have never been answered owing probably to lack of interest on this type of fundamental approach to the lac cultivation. Lac insect is a glaring example which bears the testimony to the fact that unless fundamental principles are worked out, suitable technology cannot be evolved for applying it to human welfare. This important product is a monopoly of India and can be a very important item of export in a big way if basic data on lac insect physiology especially nutrition, insect-host relationship and ecology are collected and utilized for increasing the production.

Nutrition of biological control agents is another field awaiting attention of scientists. Success of biological control also partly depends upon the ease with which parasites or predators can be reared in large numbers for releasing them in the field at proper time. To achieve this, main or alternative hosts should be available. The parasites which have high degree of specificity, do not readily accept alternate hosts. Only in certain cases mass-breeding of parasites has been possible on different host insects. *Trichogramma,* an egg parasite of several lepidopterous insects associated with sugarcane, can be mass-bred on the eggs of *Corcyra cephalonica.* This problem of alternate host insect can be solved if methods could be worked out for evolving suitable artificial diets for parasites. With the available data on insect nutrition, one can visualize that the nutritional requirement of parasites should not differ much from other insects. Some degree of success has been achieved in rearing *Pseudosarcophaga affinis* (a dipterous parasite) on a chemically defined diet (House, 1958). Parasites because of their obligatory nature have special feeding requirements which must be fulfilled to enable the parasite to establish on artificial diets. An analysis of these factors is therefore as vital as the nutritional requirement of this class of insects.

Insect nutrition as a tool in other scientific researches: Insects have been used for basic and fundamental researches pertaining the medical and genetical sciences. Indeed it was the use of *Drosophila* in fundamental genetics that ultimately led to the necessity of devising chemically defined diets to eliminate errors due to variation in food of test cultures. Similarly synthetic diets are now preferred for rearing test insects for bioassay of insecticides and for precision physiological and biochemical experimentations.

Working with *Tenebrio molitor,* Fraenkel and his associates discovered a new vitamin of B complex which was named as vitamin BT. This Tenebrio-factor was later identified as carnitine.

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22

NUTRITIONAL REQUIREMENTS OF STORED GRAIN INSECT PESTS

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Nutrition is a chemical process involving various chemical activities and physiological mechanisms by which nutrients are transformed into body materials and energy.

Knowledge of insect nutrition is of great fundamental and applied value. Importance of research in insect nutrition can be judged from a statement of House (1964), "Specifically nutritional research, advanced insect biochemistry, nutrition, dietetics, pathology, genetics, physiology and ecology and sometimes perhaps there were overtones, specially at metabolic levels of taxonomic significance. Certainly then nutritional research softened many hard entomological problems so that today many problems are better understood and nearer to solution than before."

"Nutritional requirement" has been defined as a chemical factor essential to the adequacy of ingested food (Beck, 1956). This, however, excludes the substances which are provided by symbiotic microorganisms harboured by certain insects. To include these cases House (1965) has defined this term to comprise chemical factors essential to the adequacy of absorbed nutritive material. "Chemical feeding requirements" and "physical feeding requirements" are, respectively the chemical and physical factors that are important to normal feeding behaviour. These are not considered with the nutritional requirements, though these are not less important by any means, for on these depends the selection of food in nature and the ingestion of food in the artificial diet.

Nutrients provided as food are utilized in insect body for maintenance, growth and reproduction. These three phases of activities are not similar and uniform throughout the life of an insect. For example, embryo in an insect egg develops a little and depends for its provisions on whatever is transferred from the mother in the egg and this in turn would vary from one insect species to another and would depend upon the type of reproduction and amount of yolk contained in the egg. The larval stages need food for maintenance and growth only, whereas adult stage needs mostly for maintenance and reproduction and rarely for growth. Moreover, even different larval stages of the same insect would differ in their nutritional requirement because of their different rate and phases of growth. Obviously, a particular stage of different insect species is likely to have more similar nutritional requirements than the different stages of the same insect.

Nutrients are mostly divided into two categories, namely, essential and nonessential. The criteria for this grouping cannot be fixed and also the criteria on which the essentiality or the nonessentiality of nutrient can be judged are difficult to define. Epistein (1965) discussed critically the different criteria used by several workers in the field of animal and plant nutrition and rightly concluded that the criteria of essentiality are mental constructs which, like the taxa of taxonomists, are not easy to apply unambiguously in many of the complex situations that exist in nature and therefore, it is necessary in such cases to detail the facts of matter in so far as these are known. Gordon (1959) discussed critically the criterion of adequacy of an experimental diet used by different workers in insect nutrition research and concluded that in order to avoid uncertainties, the term essential nutrients should be restricted to substances that must be present in the diet to maintain growth and reproduction indefinitely.

In order to work out the essential nutritional requirements of insects it is necessary to climinate the nonessential components of a basic diet so that a "minimal diet" is obtained. This would obviate or reduce the possibility to a minimum of interconversion of one nutrient into another when one nutrient is deleted from the basic diet each time to prepare the experimental diet. This is necessary because the interconversion of nutrients is likely to lead to a wrong conclusion (Gordon, 1959).

The other pre-requisites to nutrition research are the preparation of a *holidic* diet and raising an *axenic* culture of the organism of which nutritional requirements are to be determined. A holidic diet is one whose chemical composition is known before compounding and the axenic culture is the pure culture of identified organism.

Principles of insect nutrition

Covering the broader aspect House (1966) proposed three principles of insect nutrition, namely, the principle of sameness, the principle of nutrient proportionality and the principle of cooperating supplements. These principles can be elaborated as follows:

(i) The rule of sameness states that the nutritional requirements of insects are much the same, irrespective of their systematic position or feeding habit. The sameness of requirements in this rule relates to qualitative sameness of main classes of nutrients. The nutritional requirements of different insects may, however, differ due to the association of symbiotic microorganisms or even some insects may have special requirements. Moreover, as further knowledge in insect nutrition is gained many exceptions to this rule are likely to emerge.

(ii) The principle of nutrient proportionality states that metabolically suitable proportions of nutrients are needed for normal nutrition of an insect. This, however, does not rule out the possibility that there can be more than one optional diet for an insect.

(iii) The principle of cooperating supplements states that supplementary or substitutive sources of nutrients cooperating with the commonly recognized food stuff of the species are needed to fulfil the nutritional requirements in many insects. According to this rule when nutritional requirements of an insect are not met with from one food plant, more varieties of plants are eaten by that insect to complete the requirements.

Measurement of usefulness of food: A great deal is known about the qualitative nutritional requirements of insects in general and stored grain insects in particular. The quantitative aspects of insect nutrition are a neglected field and relatively little is known concerning the intake, digestibility and efficiency of conversion of defined diets. Quantitative work with artificial diets has usually involved only measurements of the amount of a particular nutrients required per unit of diet so that maximum percentage of adults is formed in minimum period of time. This defines the relationship between the requirements for particular nutrients but says nothing of food intake and absolute requirements or the efficiency of food utilization.

Waldbauer (1968) has reviewed this aspect of insect nutrition critically and following values have been suggested to work out the efficiency of the whole diet or a particular constituent there of:

(1) *Consumption Index*—$\text{C.I.} = \frac{F}{TA}$

where,

F=Fresh or dry weight of food eaten (gms).
T=Duration of feeding period (days).
A=Mean fresh or dry weight of animal during feeding period.

(2) *Approximate Digestibility*—

$$\text{A.D.} = \frac{\text{wt. of food ingested—weight of faeces}}{\text{wt. of food ingested}}$$

(3) *Efficiency of Conversion of Ingested food to body substance*—

$$\text{E.C.I.} = \frac{\text{wt. gained}}{\text{wt. of food ingested}} \times 100$$

(4) *Efficiency of Conversion of Digested food to body substance*—

$$\text{E.C.D.} = \frac{\text{wt. gained}}{\text{wt. of food ingested—wt. of faeces}} \times 100$$

Most of the measurements of intake and utilization of food have been made with insects feeding on plants. It is because of the great ease that uneaten food could be separated from faeces. On the contrary, practically no work has been done on the consumption of food by stored grain insects. The main reason for this is that it is impracticable or rather impossible to separate uneaten food from faeces quantitatively because of the powdery nature of uneaten flour or artificial diet on which these insects are normally grown.

In recent years markers such as chromic oxide (McGinnis and Kasting, 1964) and radioactive tracers (Crossley, 1963; Kasting and McGinnis, 1965) have been used in measuring the utilization of food by insects. These methods have the disadvantage of requiring the addition of a substance of unknown physiological effect to the diet. Further more, the use of such a marker does not obviate the need for a partial separation of uneaten food and faeces. Krishna and Saxena (1962 b) claimed to have developed an indirect method of using sugar as markers. Their method is based on the premise that ethanolic extracts of larval faeces of *Tribolium castaneum* and *Trogoderma granarium* do not contain sugars which react with anthrone. Thus they used colorimetric determination with anthrone to estimate the rate of uneaten flour in an unseparable mixture of flour and faeces by the following calculations:

$$\text{Wt. of uneaten flour} = \frac{S_2}{S_1} \times \text{wt. of flour introduced.}$$

where,

S_1=Total sugars in ethanolic extract of a sample of flour.

S_2=Total sugars in ethanolic extract of a sample of mixture of faeces and uneaten flour.

Bhattacharya and Waldbauer (1969a), however, found that the faeces of *Tribolium confusum* and several other stored grain insects react strongly with anthrone.

Recently Bhattacharya and Waldbauer (1969 b) developed a method for estimating the proportion of uneaten food and faeces in a mixture. The method depends upon the fact that the faeces contain uric acid while food does not. Thus it is possible to calculate quantitatively the amount of faeces present in a mixture by determining its uric acid contents. This method has been used successfully with *T. confusum* (Bhattacharya and Waldbauer, 1970).

Water requirement : Water requirement of stored grain insects feeding on an artificial diet has been little worked out, but the minimum water content in the natural diet necessary for their development, growth and reproduction is well known

The amount of water needed in the food of insects depends upon the rate which it is lost by the body and this depends upon the intrinsic factors like nature of cuticle, respiratory and excretory systems, and also upon an extrinsic factor like drying power of embient air. Stored grain insects living in constantly dry conditions and feeding on very dry food extract almost every trace of water from their excrement. Such insects can meet much of their water requirment by the water produced in metabolism from the oxidation of dry food.

There are striking differences in respect of requirment for water content in the food of these insects. For example, *T. confusum*, *Oryzaephilus etc.*, grow fairly well in most foods; *Ephestia kuhniella* grows even at 1% but *Lasioderma, Sitodrepa* and *Ptinus* require 10% water (Fraenkel and Blewett, 1943 a). Minimum water content in the food for *Rhizopertha dominica* and *Corcyra cephalonica* is 9-10%; *T. castaneum* and *Cadra cautella* 10%; but *Trogoderma granarium* is capable of breeding even at zero to 1.9% (Pradhan, 1969).

Protein and amino acid requirements: Proteins are essential nutrients for any animal including insects. The value of a protein in nutrition, however, depends upon the quantitative and qualitative amino acid composition and that is why in most of the investigations, requirements of amino acids have been studied rather than that of protein.

Insects require complete protein for growth. *T. confusum* larva did not grow with either zein or gliadin as the sole source of protein though casein proved better. Supplementation of zein with both lysine and tryptophan permitted as good a growth as that obtained with casein. Casein meets the amino acid requirements of *T. granarium* (Bhattacharya, 1963).

Like other insects most of the stored grain insects also need the same 10 essential amino acids as required by rats *e.g.*, threonine, tryptophan, histidine, arginine, leucine, lysine, isoleucine, methionine, valine and phenylalanine. The omission of lysine, threonine, phenylalanine, methionine, isoleucine or arginine from an amino acid mixture caused considerable larval mortality and extended developmental period in *T. confusum*; the omission of leucine, tryptophan, histidine or valine resulted in the formation of a few or no pupae, although many larvae survived the experimental period of 100 days. The omission of nonessential amino acids had no effect on the proportion of larvae reaching the pupal stage and little effect on the developmental time (Lemonde and Bernard, 1951). In *T. granarium* when protein faction (casein) was replaced by a mixture of 19 amino acids, growth and development were nearly as good as that in the normal food. But in the absence of any one of the ten essential amino acids larvae failed to grow and pupate (Pant *et. al.*, 1958; Bhattacharya, 1963). Replacement of casein or 19 amino acid mixture by a mixture of 10 essential amino acids, however, affected the development very adversely and significantly fewer adults were formed and larval period was very much prolonged, probably because of the creation of some abnormal metabolic conditions. Of the 10 essential amino acids the deficiency symptoms of valine and isoleucine were most striking and in their absence there was high larval mortality and the surviving larvae had very stunted growth. Insects fed on diet lacking one of the nonessential amino acids at a time developed normally when proline or hydroxyproline was deleted. But on the omission of alanine, cystine, serine, aspartic acid or glutamic acid much fewer adults were formed; glycine occupied an intermediate position and deficiency of tyrosine caused most marked ill effects (Bhattacharya and Pant, 1968 a). Contrary to this, Davis (1956) showed that the omission of arginine, tryptophan, isoleucine, histidine or methionine (essential group), cystine or glycine (nonessential group) from the artificial diet of *O. surinamensis* resulted in poor growth and development; quicker development and more larval survival were possible in the presence of definite quantity of lysine (Davis, 1961) and arginine (Davis, 1962).

O. surinamensis needed tryptophan, phenylalanine and tyrosine for normal development (Davis, 1961 a); the optimal concentration of tryptophan was 3 mg/gm of food and it could not replace phenylalanine and tyrosine even when it was present at a concentration of 10 mg/gm of diet (Davis, 1968b). These two amino acids could be dispensed with if arginine was present at an optimal concentration of 9 mg/gm of diet. This showed that alanine and proline were used for the synthesis of arginine if this amino acid was not present in the sufficient quantity in the diet (Davis, 1968 c). In a later study, Davis (1969) showed that in this insect glycine serves as a precursor of serine. *Tenebrio molitor* requires about 10-12 amino acids as essential nutrients in the artificial diet for growth and development (Leclercq and Lopez-Francos, 1964).

Some iodoamino acids and iodoproteins, though certainly not essential had some beneficial effects on growth and development of *C. cephalonica* (Moudgal *et. al.*, 1958).

Amino acid requirement of insects can also be influenced by intracellular microorganisms. In *Stegobium paniceum* omission of any one of the nonessential amino acids had no effect on the growth of larvae except in case of glycine, while removal of any one of the 10 essential amino acids resulted in marked disturbance in growth and development except in case of tryptophan and probably phenylalanine. But in aposymbiotic larvae omission of one single amino acid resulted in very poor growth and prolonged larval period (Pant *et al.*, 1960). On the other hand, omission of any one of the amino acids except arginine and histidine did not hamper growth and development of *Lasioderma serricorne* probably because of the amino acid synthesis by microorganism (Pant and Kapoor, 1963 a).

From the above discussion it can be concluded that 10 amino acids are generally essential unless symbiotic microorganisms synthesize a few of them. Besides these, other amino acids may or may not be required for normal growth and development in certain species depending upon the metabolic capabilities of the insects concerned particularly with reference to mechanisms that enabled one substance to be substituted for another.

Lipid requirements: Insects apparently are highly capable of utilizing fats and when necessary can synthesize these from proteins and carbohydrates. Although most insects probably synthesize all the fatty acids required, a few species are known to require dietary sources of certain unsaturated fatty acids (House, 1965).

Fraenkel and Blewett (1945, 1946) demonstrated that wheat germ oil was needed for the normal development of *E. kuhniella*. The critical substance in the wheat germ oil was identified as linoleic acid. This unsaturated fatty acid is needed for the normal formation of adult scales and is perhaps necessary either for the production of moulting fluid or for its proper functioning because in the absence of linoleic acid the caterpillars of *Ephestia* did reach pupation but adult moths failed to emerge. Linoleinate was equally effective but oleate was an inadequate substitute indicating that this insect could not convert oleate to linoleinate. Peculiarly enough, the other species of *Ephestia* and *Plodia interpunctella,* closely related moths could not be shown to require any dietary fatty acids. *Tenebrio* larvae however could synthesize linoleic acid (Fraenkel and Blewett, 1946, 1947).

Singh (1955) reported that *C. cephalonica* did not require any fatty acid but Oberoi (1956) showed it convincingly that linoleic acid was essential for the growth and development of this insect. *Trogoderma* grubs failed to utilize linoleic acid though their growth was almost normal on artificial diet containing arachidic acid as a source of lipid (Pant and Pant, 1961). Palmatic and oleic acids individually were most effective dietary lipids although a mixture of linoleic, oleic and palmatic acids resulted in most rapid development and best survival of *Oryzaephilus* larvae (Davis, 1967).

A dietary sterol appears to be essential for growth and development in most of the insects for, unlike mammals, they are incapable of synthesizing sterols from simple precursors. The apterygote *Ctenolepisma* is unusual in being able to synthesize labelled cholesterol from C^{14} acetate (Clayton *et al.*, 1962).

Usually cholesterol is best utilized by most of the insects, although its short chain derivatives 7-dehydrocholesterol, ergosterol, sitosterol and certain phytosterols may be satisfactory for many species. The nutritive value of various sterols is tabulated on next page.

Cholesterol acetate, esterate and β- sitosterol were also utilized by *Trogoderma* larvae (Agarwal, 1970). The normal larvae of *L. serricorne* grew well on a diet deficient in cholesterol but the aposymbiotic insects behaved differently and indicated the essentiality of cholesterol. It showed that yeast-like symbiotes in the insect synthesize and supply cholesterol for good growth and development of this insect (Pant and Kapoor, 1963 b).

The principal sterols required by *T. confusum* larvae are cholesterol and 7-dehydrocholesterol. Smissman *et al.*, (1964) isolated and characterized dehydroepiandrosterone, pragnanolone and progesterone as products of normal metabolism in these insects. This indicated that *T. confusum* possessed the enzymatic capacity to alter the side chain of dietary sterols present in yeast contained in the diet. These investigators tentatively identified androstenedione as well and proposed the following scheme for sterol interconversion in this insect.

$$\begin{tikzcd}
 & \text{Ergosterol} \arrow[d] & \\
 & \text{Cholesterol} \arrow[dl] \arrow[dr] & \\
\text{Dehydroepiandrosterone} \arrow[d] & & \text{Pragnanolone} \arrow[d] \\
\text{Androstenedione} & & \text{Progesterone} \arrow[ll]
\end{tikzcd}$$

Whether any of these compounds which play an important role in mammals have any physiological role in insects remains to be known.

Carbohydrate requirements: In most of the insects carbohydrates are not essential dietary substances, but a few need carbohydrate for growth and development. Some of these exceptions occur among species that live on foods rich in carbohydrates and include, for instance, certain stored products insects. The larvae of *Tenebrio molitor* did not grow on a diet containing less than 40% carbohydrate and best growth was obtained on diets containing 72% carbohydrate (Lafon and Teissier, 1939). Beetles of the genera *Oryzaephilus, Stegobium* and caterpillars of the moth, *Ephestia* grew slowly and failed to pupate when fed on a diet low in carbohydrate. Similarly, the growth of *Tribolium, Lasioderma* and *Ptinus* was accelerated in the presence of carbohydrate (Fraenkel and Blewett, 1943 a). *Corcyra* larvae required 80% glucose for optimum growth (Singh, 1955). All the same, stored grain

NUTRITIVE VALUE OF SEVERAL STEROLS IN INSECTS

Sterols	*Tribolium confusum* (Fraenkel and Blewett, 1945)	*Laisoderma serricorne* (Fraenkel and Blewett, 1945)	*Stegobium paniceum* (Fraenkel and Blewett, 1945)
Cholesterol	+++	+++	+++
7-dehydrocholesterol	+++	+++	+++
Cholestanol	++	++	++
Sitosterol	+++	+++	+++
Stigmasterol	—	—	—
Stigmastanol	—	—	—
Epicholestanol	—	—	—
Zymosterol	+	+	++
Ergosterol	+++	+++	++
	Ephestia kuhniella (Fraenkel and Blewett, 1946)	*Tenebrio molitor* (Leclercq, 1948)	*Callosobruchus chinensis* (Ishii, 1951)
Cholesterol	+++	+++	+++
7-dehydrocholesterol	+++	—	—
Cholestanol	+	—	+++
Sitosterol	+++	+++	+++
Stigmasterol	—	+++	+++
Stigmastanol	—	+++	—
Epicholestanol	—	—	++
Zymosterol	O	—	—
Ergosterol	++	+++	—
	Oryzaephilus surinamensis (Fraenkel, 1959)	*Ptinus tectus* (Fraenkel, 1959)	*Trogoderma granarium* (Pant and Pant, 1961 ; Agarwal, 1970)
Cholesterol	+++	+++	+++
7-dehydrocholesterol	+++	+++	+++
Cholestanol	++	++	+++
Sitosterol	+++	++	+++
Stigmasterol	—	—	+++
Stigmastanol	—	—	—
Epicholestanol	—	—	—
Zymosterol	++	+	—
Ergosterol	++	+++	++

N.B.—Number of + signs indicates degrees of utilization ; O = not utilized ; — = not studied.

insects do not show any great specificity for particular carbohydrates as many kinds except pentoses, may be utilized advantageously by the larvae and the adults. *T. molitor* larvae developed rapidly on starch, mannitol and levulose; glucose, sucrose, xylose, lactose and glycogen gave slow growth; arabinose gave maintenance only; with galactose or inulin the larvae lost weight (Lafon and Teissier, 1939). For the growth of larvae of *T. confusum* sucrose was the most effective source; glucose, mannose, maltose, raffinose, white dextrin, starch, mannitol and sorbitol were nearly equally suitable; lactose, melibiose, rhamnose and inulin supported growth but at a slower rate, whereas with xylose, arabinose levulose, galactose, or yellow dextrin none of the larvae reached pupation (Bernard and Lemonde, 1949). *T. castaneum*, a closely related species to *T. confusum* differed from the latter that it could not use rhamnose, inulin and sorbitol at all; sucrose was comparatively less used but galactose much more effectively (Pant and Gabrani, 1963 a; Pant and Dang, 1965 b). Larvae of *Stegobium* failed to utilize galactose, xylose, arabinose and those of *Oryzaephilus* could not use xylose, sorbose, dulcitol and inulin (Lemonde and Bernard, 1953). *T. granarium* larvae failed to utilize pentoses namely, arabinose, xylose, rhamnose and sorbose; glucose, maltose, sucrose and soluble starch were more suitable; diets containing melibiose, cellobiose, lactose, raffinose, melizitose or trehalose gave poor growth. Sugar alcohols like mannitol and dulcitol could not support the larval growth (Pant and Oberoi, 1958).

Carbohydrate-hydrolysing enzymes namely, amylase, β-glucosidases, galactosidases and invertase were reported from the alimentary canal of the larva and adult of *T. castaneum* (Krishna and Saxena, 1962 a) and larva of *T. granarium* in which β-glucosidase was absent but β-fructosidase was found to be present (Krishna, 1958).

Vitamin requirements: Vitamins are essential accessory growth factors required by most of the insects and their quantitative requirement for insects is very little. All the same these are indispensable metabolically.

Most of the vitamins required by insects belong to the B-group comprising thiamin (B_1, aneurine), riboflavin (B_2), nicotinic acid, pyridoxine, pantothenic acid, choline, inositol. p'aminobenzoic acid and biotin. *For T. confusum* riboflavin, nicotinic acid, biotin and pantothenic acid are indispensable; the growth rate is adversely affected if any one is lacking. Choline and pyridoxine have somewhat less effect but are still important. Inositol and p^1-aminobenzic acid are of minor importance (Fraenkel and Blewett,1943b, 1946) *P.tectus* requires all the vitamins of B-group, whereas *T. molitor, E. kuhniella* and *P. interpunctella* require all except inositol and p^1-aminobenzoic acid (Fraenkel and Blewett, 1943 b). *S. paniceum* required biotin and thiamin (Lemonde and Bernard, 1953). *L. serricorne* and *S. paniceum* required thiamin and pyridoxine but the aposymbiotic forms of these insects required riboflavin, niacin, pantothenic acid, choline, biotin and folic acid (Pant and Fraenkel, 1954). These workers convincingly showed that symbiotic microorganisms synthesize and supply major vitamins of B-group to these insects.

O. surinamensis larvae required riboflavin, pantothenic acid, choline chloride and nicotinic acid while thiamin or folic acid omission produced less drastic deficiency symptoms (Kolya and Pant, 1961). *T. castaneum* essentially required riboflavin, nicotinic acid and pantothenic acid; deficiency of pyridoxine, folic acid or biotin also hampered metabolism and growth to some extent; choline, thiamin and p-aminobenzoic acid were of no importance. Inositol appeared to be important in the development of male larvae of *T. castaneum* (Pant and Gabrani, 1963 a, b; Pant and Dang, 1965 a). *T. granarium* is susceptible to choline, nicotinic acid or pantothenic acid deficiencies and these were required at the rate of 200, 15 and 10 microgram per gm of diet respectively (Pant and Ghai, 1959). Using purified casein, Pant and Pant (1960) could not confirm the essentiality of choline, though besides nicotinic acid and pantothenic acid, riboflavin and folic acid were found important and thiamin was also of significant value.

Certain additional accessory factors are also required by insects. In addition to the 8 vitamins of B-group and folic acid *Tenebrio* required a new factor, B$_T$ which later was identified as carnitine (Carter *et al.*, 1952; Fraenkel, 1959). Three factors influenced carnitine deficiency in *T. molitor*, namely the sample of the casein used, the strain of the insect and the co-existence of deficiencies of zinc and potassium (Fraenkel, 1958). Carnitine is almost universal in living tissues but nothing is known of its function. Like choline it may perhaps be a constituent of phospholipid (Wigglesworth, 1965). Some insects are able to synthesize carnitine and do not require it in the diet but *Tribolium* is quite sensitive to its absence (Naton, 1963; Pant and Gabrani, 1963 b).

Minimal quantitative requirements for vitamins can only be known in relative term depending upon the relative concentration of other components in the diet. For example, the minimal level of nicotinic acid required for optimal growth of *T. confusum* was 4 microgram per gram of diet on 5% casein, and 64 microgram on 75% casein; whereas *T. molitor* needed 8 micrograms on 5% casein and 128 micrograms on 50% casein (Fraenkel and Stern, 1951).

Owing to vitamins acting generally as constituents of enzymes essential in metabolic activities, their deficiency may adversely affect activities of insect. In the larvae of *C. cephalonica* thiamin deficiency resulted in the accumulation of large amount of pyruvic acid in their tissues as happens in vertebrates (Sarma and Bhagvat, 1942) and degenerative changes may occur in the cellular contents of the muscular, adipose and midgut epithelial tissues (Swamy and Sreenivasaya, 1942). Similarly in *T. confusum* thiamin deficiency produced fat body with small cells and other degenerative symptoms. On biotin-deficient diets tissues of *C. cephalonica* larvae did not desaturate palmatic acid and stearic acid as effectively as those of larvae fed biotin, nitrogen accumulation in tissues was relatively higher and that of fat and cholesterol relatively lower on the deficient diets (Sivasankar and Sarma, 1951, 1952). Moreover, pyridoxine deficiency upsets tryptophan metabolism and led to a block in the conversion reaction between kynurenine and 3-hydroxykynurenine or 3-hydroxyanthranilic acid (Sundaram and Sarma, 1953; Shanmuga Sundaram and Sarma, 1954). The action of B_{12} in overcoming zinc toxicity in *C. cephalonica* was supposed to be due to its possible involvement in nucleic acid synthesis (Sivaramasastry *et al.*, 1958). According to Fraenkel and Chang (1954) lack of carnitine in *T. molitor* impaired the regulatory system that controls water loss. Severe histopathological effects occurred in oenocytes, Malpighian tubules, haemolymph and fat body but not in neural and muscular system.

Mineral requirements: The various minerals which are found in insects take part in regulation of osmotic pressure of the intra-and extra-cellular fluids in the maintenance of an ionic balance suitable for the activity of the living cells, and as co-factors in some enzyme systems and as integral portion of the others.

Investigation of mineral requirements in insect nutrition is the most neglected area of research as well as a difficult one to work with. The reasons for this are: firstly, it is difficult to prepare diets free of active levels of a number of mineral elements; if trace amounts of these occur as impurities in one or the other of the main dietary ingredients these may mask negative growth responses in classical deletion experiments. Secondly, because mineral nutrients are usually provided as salts; elimination or changes in the concentration of particular ion will automatically involve some change in the concentration of another ion. For example, if magnesium is provided as chloride its omission entails less of chloride to compensate for which may require the introduction of more of a third ion such as calcium as calcium chloride and so on. This makes the adjustment of total mineral mixture complicated if one is to be confident that effect on growth of omitting a particular ion is due specifically to its omission alone rather to concomitant change in the level of other ions (Dadd, 1968). Thirdly, no rational salt mixture specifically designed for insects is available and in most of the studies one or the other salt mixture developed for vertebrate feeding has been adopted. This is most illogical, for, such salt mixture cannot be well balanced for insect requirment. The reasonable basis on which an ideal salt mixture for insects can be designed is the knowledge of inorganic chemical composition of insect body or of its natural food.

Quantitative mineral requirements have been worked out in a few insects. The larvae of *T. confusum* required phosphorus (Nelson and Palmer, 1935) and were insensitive to phosphorus changes over a wide range, optimum level varying from 0.125 to 0.475%. The effects of phosphorus deficiency were evident at 0.075% while toxic effects were observed at 3.75% (Chaudhary and Lemonde, 1962). Potassium and magnesium were absolutely essential for the development of this insect; deficiency of calcium in the diet caused rapid dehydration of pupae and suppression of adult emergence (Huot *et al.*, 1957, 1958; Medici and Taylor, 1966). Medici and Taylor (1967) also worked out the interrelationship among copper, zinc and cadmium in the diet of confused flour beetle. Additional copper slightly reduced the toxic effect of high level zinc and cadmium, and higher levels of zinc reduced cadmium toxicity. The possible mechanism for this was imagined to be the competition for enzyme sites.

Phosphorus, potassium and magnesium were found essential for the development of *T. castaneum* (Pant and Dang, 1967); potassium and zinc were indispensable for *T. molitor* and their deficiency in the diet cause carnitine deficiency (Fraenkel, 1958). At high levels zinc was toxic to *C. cephalonica* by decreasing catalase activity (Sivaramasastry *et al.*, 1958).

Physiology of mineral nutrition in *T. granarium* has been worked out in a greater detail than in any other insect. In a preliminary report Pant *et. al.*, (1969) reported that in the absence of a mineral mixture *Trogoderma* grubs could not develop at all and they tolerated as high a salt content as 28.17% in the artificial diet. Misra (1970) extended this study further and confirmed the findings of earlier workers regarding the essentiality of inorganic salts in the artificial diet. The optimum quantity of salt mixture required was 8.93% and as high as 42.37% was tolerated. While explaining the mechanism for high salt tolerance it was shown that the grubs did not leave out salt particles selectively but higher salt content in the diet retarded the rate of feeding probably due to less frequent emptying of foregut. Increasingly more amount of salt was retained in the body as the salt content exceeded 16.39% of the diet. Potassium and magnesium were also absolutely essential and the optimum quantity of magnesium sulphate and monopotassium phosphate required to meet the demand for magnesium and potassium was 0.54 and 2.4 per cent respectively; deletion of calcium from the diet also affected development adversely. Potassium stimulated feeding and more amount of protein was synthesized and increasingly more amount of labelled dl-leucine was incorporated in the body protein as the potassium phosphate in the diet was increased to 2.04. Sodium, iron, zinc and manganese were probably not needed. Of the anions, phosphorus was absolutely essential but deletion of sulphate and chloride did not affect the development at all.

Role of symbiotic microorganism: Mycetomes containing hereditary symbiotic microorganisms have been reported from *Stegobium* and *Oryzaephilus* (Koch, 1933, 1936) and *Calandra* and *Rhizopertha* (Mansour, 1934). The contribution of microorganisms in insect nutrition has been determined by comparing the nutritional requirement of a normal insect with that of a sterilized one.

The yeast isolated and cultured from *Stegobium* and *Lasioderma* are morphologically characterstic and distinguishable. These are not host specific and are interchangeable in these two species. These have been proved to be a rich source of the vitamins of B-group and sterols (Pant and Fraenkel, 1950, 1954); these probably supply their hosts with essential amino acids (Pant and Kapoor, 1963 a, b). In pure culture the yeast of *Stegobium* released thiamin, riboflavin, nicotinic acid, pyridoxine, pantothenic acid, folic acid and biotin into the medium (Grabner, 1954). These are precisely the vitamins with which the insects with symbiotes could dispense. This suggested that in the normal course of events vitamins from the symbiotes reached the host by diffusion from mycetomes. The symbiotes of *Calandra* supplied some unidentified nutrients but it was distinct from any of the vitamins needed by *Tribolium* (Schneider, 1956).

Natural food: It is a common knowledge that insect pests associated with stored grains do not feed, breed and develop equally well on all the grains and there is certain amount of host specificity. There is hardly any experimental evidence for such a specificity though the possible reasons for this could be the absence of phagostimulants, deficiency of some essential nutrients, imbalance of nutrients or the presence of some growth inhibitory factors like enzyme inhibitors *etc.*, some physical factors like hardness, shape of seed, thickness and texture of seed coat may also be involved. A search for such factors will be of great economic and practical value.

T. granarium which attacks large number of grains shows more preference for cereals and millets than for pulses. Bhattacharya and Pant (1968b) showed that larvae of *T. granarium* were unable to penetrate the whole grain of any of the nine pulses tested. In flour form pulses fell into three main groups. On flours of green, black, Bengal and Kabuli gram, cowpea and pigeon pea development was as successful and rapid or nearly so as on wheat flour and showed little improvement when the yeast was added. Development on soybean and lentil flours was very slow and showed marked improvement when yeast was added. By adding increased proportions of balanced diet it was shown that performance of larvae on soybean, lentil and even on French been flours could be improved but with the last two it was not so good as even on cellulose. It was, therefore, concluded that lentil and French bean lacked some essential nutrients and might also contain growth inhibitors.

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NUTRITION OF PHYTOPHAGOUS INSECTS

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Research on insect nutrition has passed through a transition phase from a descriptive naturalist qualitative stage to a more fundamental or quantitative stage. Till the year 1930, much of the work consisted of observations on the growth of insects on various foodstuffs that are more or less accessible to the insect in nature. The complexity of natural foodstuffs, limits their usefulness in precise determination of the essential ingredients required by the insect. Much of the difficulty has been overcome after 1940 onwards by the availability of highly purified, chemically definable synthetic diets which can be altered in composition for almost any purpose hence their popularity in fundamental nutritional studies is increasing. The importance of artificial diets for insects has grown further with the growing interest in insect physiology and insect toxicology.

Insects with omnivorous food habits and with adaptability to laboratory culture have been used for many years as test insects for the study of nutrition. The nutrition of plant feeding insects has not been studied with much success until recently. In devising artificial diets for these insects, radical changes in composition and physical properties from the natural food are bound to occur. Most of these insects are highly selective in their food habits, choosing only a single species of plant or a family of plants or particular part of the plant.

The first successful attempt to rear a phytophagous insect on an artificial diet was made by Bottger (1943) who compounded a diet for the European corn borer *Pyrausta nubilalis*. It consisted of casein, sugars, fat, salts, vitamins, cellulose, agar and water. The diet was later on utilized by Beck *et al.*, (1949) at the University of Wisconsin, in their studies on nutritive requirement of this borer. The diet was composed of pure chemicals and highly purified natural products but included a corn leaf extract to provide unidentified growth factors. Later on many other investigators modelled their experimental diets after the corn borer-diet, which was also known as "Beck's" diet at those times. Ishii (1952) and Matsumoto (1954) used this diet with extracts of the host plants for the Asiatic rice borer *Chilo suppressalis* and the oriental fruit moth *Grapholitha molesta*. Vanderzant and Reiser (1956) were able to rear aseptically the pink bollworm *Pectinophora gossypiella* on a diet without any plant extract. Since these early experiments, a number of insects have been reared on diets consisting entire of pure chemicals and other nutritive substances that are completely foreign to natural food of the test insects.

To do the best work in the nutrition one should use chemically defined diets under asceptic conditions. Microorganisms must be excluded because they, through metabolic processes, can produce almost every dietary factor, consequently ruining the significance of the nutritional test.

In insects which feed on stored products, formulation of the artifiicial diet is easier. They do not need much water as only 10% water is enough for their growth and development. It has been found that microorganisms do not grow in diets containing only 10% water. Further, physical feeding requirements in most of the stored product insects are also simple. They feed on powdered diet except those few ones like *Sitophilus* sp. and *Bruchus* sp. which need a solid surface of whole grain to lay eggs on. For such insects powdered diets are made into artificial pellets.

Formulating a suitable chemically defined diet for a plant feeding insect is much more difficult problem than those which feed on stored products. In general they require 70-80% water in the diet which is more liable to be contaminated by microorganisms. Physical requirements for feeding are also numerous and differ in different species. In phytophagous insects a chemically defined diet should fulfil the following requirements: (i) it must satisfy the insect's chemical attractiveness; (ii) have a satisfactory physical condition; (iii) must be free from contaminations and (iv) must contain a correct amount of nutritional elements needed for normal growth.

Chemical attractiveness—A diet that has an adequate amount of nutrients may fail to support growth of an insect if the stimuli that initiate feeding are lacking. There is controversy whether odd plant substances (Fraenkel, 1959) or nutritive materials solely determine food preference in insects. Generalization of either of the two extremes is certainly not justified. Examples are now known in which either of the two groups of substances or even a combination of these two, play a crucial role. Though there is some evidence that non-nutritive chemicals cause feeding response in certain leaf-feeding insects, the successful rearing of many phytophagous species without the incorporation of plant extracts indicates that most insects can be reared on chemically defined media. Chemical feeding-stimulants are frequently supplied by the nutrients. Sugars are perhaps the most important feeding stimulants for phytophagous insects. Beck (1956) found that the feeding of

Pyrausta nubilalis larvae was stimulated by glucose and that fructose and sucrose were also stimulatory. Ito (1960) found that *Bombyx mori* larvae were stimulated by sucrose and little stimulation resulted from glucose. Adults of *Anthonomus grandis* also preferred sucrose to glucose (Vanderzant and Davich, 1961). Scheel *et al.*, (1957) found that starch stimulated the feeding of *Oncopeltus fasciatus* whereas glucose, fructose and sucrose did not. Sugars also affected feeding in locusts. Sucrose was definitely phagostimulatory to aphids (Mittler and Dadd, 1963).

Proteins and amino acids also stimulate feeding (Davis, 1965). Some insects like the seed-corn maggot are able to discriminate between different protein sources (McLeod, 1964). In silkworm, Ito and Arai (1965) have found that an amino acid diet evokes a feeding response. Often the feeding response of insects is intensified by combining these with some other substances such as sucrose (Mittler and Dadd, 1964 a; Herron, 1965) or phosphate buffer (Robbins *et al.*, 1965).

Lipids and related compounds have also been found to stimulate feeding in phytophagous insects. Wheat germ oil (Dadd, 1960) and soybean oil (Ito, 1961) enhance the feeding activity of locusts and silkworm respectively. β-sitosterol is a phagostimulant for the silkworm (Hamamura *et al.*, 1961; Nayar and Fraenkel, 1962).

Physical conditions of diet—An artificial diet may contain all nutrients and the proper feeding stimulants but the physical form of the diet in which it is presented to the insect is also an important factor. In plant feeding insects with chewing mouth parts the physical modification of diet is frequently accomplished by adding cellulose as an inert carrier because it is not digested by most insects. Its main purpose is to add texture to the diets. It also provides roughage which aids the passage of food material through the gut (Neville and Luckey, 1962).

Most plant feeding insects require a high content of water in their diets. In natural feeds the water is retained within cell walls and bound to cellular constituents. However, in artificial diets, a nutritionally inert substance is required that will bind water and also make the diet hard. The polysaccharide agar is the preferred substance because it is compatible with dietary ingredients and can be obtained in highly purified form which makes it satisfactory for nutritional experiments. Agar forms a firm gel at concentration of 1.5% or more depending on the water content and nature of the dietary ingredients. Insects which need a firm surface against which they can press their mouth parts, may need higher quantity of agar up to 3%.

Sodium alginate is a gum which has also got a thickening property in artificial diets. Alginate reacts irreversibly with calcium ions to form a firm gel structure. Binding of calcium does not affect the growth of insect as calcium requirement of insect is very low; moreover, there is excess of calcium salts in most insect diets. However, alginate also reacts with proteins, lipids and other cations. If the reaction is irreversible the development of insect will be retarded.

There are a number of conditions for specific insects such as presence of cracks and holes, raised areas on the surface, curvature of the surface, that could also be considered as physical aspects. Vanderzant and Davich (1961) reported that shape of the diet was important in inducing oviposition in the boll weevil *Anthonomus grandis*. Up to twice as many eggs were collected if food was cast in the form of cylindrical pieces than obtained from diets with a flat surface.

Not much work has been done till recently on plant sucking insects which require a liquid diet. The major breakthrough in the nutrition of plant sucking insect was accomplished by Mittler and Dadd (1962, 1963) working with *Myzus persicae*. Auclair and Cartier (1963) gave complete chemically defined artificial diets for *Acyrthosiphon pisum*. Since then our knowledge of the nutrition of other species of aphids such as *Macrosiphum euphorbiae* (Cartier and Morin, 1965), *Aphis fabae* (Dadd and Krieger, 1967), *Aphis gossypii* (Auclair, 1967 a, b) and *Neomyzus circumflexus* (Ehrhardt, 1968 a, b, c) has considerably increased.

Nutritional work has been done also on plant sucking insects other than aphids *e.g.*, *Lygus hesperus* (Auclair and Raulston, 1966; Raulston and Auclair, 1968), *Oncopeltus fasciatus* (Srivastava and Auclair, 1970) and *Planococcus citri* (Gothilf and Beck, 1966).

Liquid diet cannot be offered to sucking insects as such. There must be some type of membrane between the diet and the insect for the stylets to probe in. The design of suitable membranes for plant sucking insects was a problem until Mittler and Dadd (1962, 1963) and Auclair and Cartier (1963) succeeded in feeding aphids through parafilm membrane. Many types of feeding cages were described by various workers. But the simplest and now most widely used one was described by Mittler and Dadd (1964 b). There is a glass tube (5×3 cm approximately) open at both ends. The membrane used is parafilm 'M', a commercially available material made by Marathon. American, Can Co, Menasha, Wisconsin, in the form of sheets and consisting of a homogenous blend of rubber and wax. Small square of membrane is taken and stretched by hand in two directions at right angles to each other, almost to a breaking point. One open end of the glass cage is covered by the pulled membrane. The membrane adheres to the rim and outer surface of the chamber. A drop (0.1 to 0.2 ml) of a diet is then placed on this feeding membrane. A second piece of parafilm 'M' membrane is then stretched and pulled down over the first and thereby sandwitching the drop as a thin layer between them. A drop may thus be made to cover the entire feeding surface but some care and practice are needed to avoid squeezing it out over the rim of the chamber. Since the two membranes adhere to each other the rim of the chamber where these come into contact, a seal is formed around the drop which prevents its evaporation and subsequent leakage. The insects are introduced through the other open end of the chamber and cork is inserted into the base of the chamber. This type of cage was used for *Myzus persicae,* and a modified cage for the studies on *Lipaphis erysimi.* In the course of investigation it was found that *L. erysimi* require more humidity inside the feeding chamber than *Myzus persicae;* so a pin with moist pad was inserted in the cork at the base of the chamber. A holed cork with fine muslin cover should be used so that the moisture may not deposit on the walls of the glass chamber, (Pant, unpublished data).

However, pH of the diet also influences growth, reproduction and survival of aphids on artificial diets (Auclair, 1965, 1967a; Cartier, 1968), *Acyrthosiphon pisum, Aphis gossypii* and *Macrosiphum euphorbiae* need slightly alkaline diets (pH 7.3 to 7.5). Further, aphids could discriminate between the difference of 0.3 to 0.6 of one pH unit.

Prevention from microbial contamination—Exact nutrient requirement can be determined only in the absence of microorganisms which may cause changes in the diet. Sterilized diet is therefore used in nutritional investigations. Diets can be sterilized by autoclaving at 120° C under 15 lbs pressure for 15 minutes. Heat liable substances such as amines and aldehyde groups of sugars and vitamins are sterilized by filtration through special filters. The pH of the mixture and protective action of other dietary components like lipids and proteins influence the amount of decomposition of heat-liable substances. Certain chemicals like sorbic acid, propionic acid, methyl paraoxybenzoate and formaldehyde *etc.,* can also be used for preventing growth of microorganisms in the diet.

To free egg surfaces from microorganisms that cause contamination a number of chemicals have been used. The choice of chemicals depends on the type of eggs to be treated.

Nutrient concentration—In phytophagous insects qualitative requirement of nutrients differ in different species. Study of qualitative requirements provide only an elementary understanding of the nutrition of an organism. Some balance is needed between the main classes of nutrients, such as proteins, carbohydrates, lipids, vitamins *etc.,* for optimum nutrition in insects. Quantitative requirement for each nutrient and consequently required balance of nutrients, can vary within and between species owing to many factors, including the synthetic abilities of the organism, and metabolic activities involving specific interrelations between certain nutrients (House, 1965).

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24

PHYSIOLOGY OF SYMBIOTES IN INSECTS

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When a microorganism accidently enters the system, for example through the wounds of an animal, it usually creates a disturbance there. The defence machinery of the animal becomes active, antibodies are produced and the host tries to eliminate the intruder. If on the other hand the microbe gets an upper hand, it multiplies in the host body, producing disease symptoms and in extreme cases, the host dies. Insects like any other animal are liable to get infected by bacteria, fungi *etc.*, which often produce diseases amongst them. Under special conditions some insect species have permitted the microbes to remain in their bodies and entered into a state of symbiotic relationship with the intruders. Such a relationship is often termed as symbiosis (*Symbios=partner*). The organs harbouring the symbiotic organisms or *Symbiotes* are called *mycetomes*. All the examples of present day symbiosis in insect is the outcome of an association which probably began millions of years ago in earlier geological era when insects were evolving.

Insects are quite unique in having symbiotic relationships with bacteria, fungi, yeast and even protozoa. The species which harbours the beneficial microorganisms is called *symbiotic* species. The one without them is *asymbiotic*. If a symbiotic species is artificially deprived of its symbiotic organisms, it is termed as *aposymbiotic*. Besides insects, the examples of such relationship are also found among some plants, luminous organs of molluscs, fish, excretory organs of annelids, but no where such a diversified and highly evolved relationship exists as that in insects. Symbiosis may occur between two plants *e.g.*, fungus and alga which together only can produce lichen pigments; or between leguminous plants and their root-nodule bacteria which fix atmospheric nitrogen for the host. The symbiosis is also found, though rarely, between animal and animal *e.g.*, flagellates and termites, or ciliates and cattle. Most widely occurring symbiosis is between animal host and microorganisms. It is the last type of symbiosis that shall be discussed in the following pages.

Symbiotes and pathogens

By the strict sense of definition of symbiosis, host-parasite relationship also comes under this category because parasitism is also the involvement of two organisms into partnership. It is therefore, necessary to understand the basic difference between symbiotic and parasitic mode of associations. Although both types of microorganisms make an abode inside insect body and both derive protection from external adverse conditions, yet the ultimate effect in the two instances is quite different for the hosts, due to the difference between the physiological behaviour of the symbiotes and parasites (pathogens). In case of parasitism the relationship leads to an attack by the invader which stimulates the defence mechanism of the host, to kill the invaders and terminate the relationship. There is no adaptation for mutual benefit and the behaviour of the parasitic microorganism is not under the control of the host.

In the symbiotic relationship a similar physical association exists but microorganism is not an invader but becomes an integral part of host body. The host provides special organs, the mycetomes for harbouring the 'guests' and permits them to even infect the newer generation in the early stage of oogenesis or at the initiation of feeding stage of the immature forms. The symbiotes in return provide nutrients like vitamins, essential amino acids, sterol and fix free nitrogen from air for the host. The host keeps a complete control over the numerical strength of the symbiotes.

Buchner (1965) has given a definition of endosymbiosis as "a community of life, governed by natural laws, and proceeding without marked disturbance; a community between two different partners, one of whom is received into the body of the other, usually a higher organised animal. Mutual adaptation has here attained so high a degree of intimacy, that we are justified in assuming this arrangement to benefit the host". The relationship is longer and even of very permanent nature so much so that internal organs of the hosts are suitably modified to ensure that each individual egg produced is infected with the microorganism characteristic of the species in question. Symbiotes normally do not undergo adaptive degeneration so characteristic of certain endoparasites.

Evolution of symbiosis: The present day symbiotic relationship between insect and microbes is so well balanced that a delicate, yet highly complex equilibrium between the two partners exists. The host's internal organs and its physiological processes have undergone several evolutionary modifications which must have taken several thousands to millions years to reach the degree of adaptations as are seen in present day insects species. Termites and blattids contain bacterium-like symbiotes which were probably acquired by their ancestors and then transmitted to all later genera and species. The representatives of nearly .10 families of blattids have a very great similarity in the nature, development and transmission of their symbiotic bacteria and in all cases mycetomes are located in the fat bodies. It is assumed that in the lower carboniferous period the bacteria were already associated with ancestors of these insects. Further, a most primitive termite family Mastotermitidae has a bacterial symbiosis, similar to that of blattids. This similarity agrees well with the fact that Blattoidea and Isoptera are closely related phylogenetically. The common ancestors of cockroaches and termites must have lived symbiotically with the same bacteria which we find in present day blattids and *Mastotermes*.

Some workers believe that the wide scattering of symbiotes among diverse groups of insects is an evidence that the symbiosis has probably arisen many times during the course of evolution. Buchner argued that symbiosis is quite different from parasitism and parasitic relationship in the course of time cannot change into symbiotic association. This theory of origin of symbiosis cannot be accepted without reservations. Perhaps parasitism and symbiosis are two extremes which result from a series of intermediary steps. When a microbe enters the host system several things may happen such as a complete destruction of the invader, or destruction of the host or both may survive in which the host is able to tolerate the microbe without any initial disadvantage. The last mentioned condition is often found in patients with chronic infections which they carry throughout their lives. Even in a pathogenic condition, the virulence of a parasite can vary according to its biological race and reaction and the biotypes of the host. In certain arthropod vectors (*e.g.,* ticks) the disease-causing organisms (*Babesia* sp.) are transmitted from one generation to another. These vectors seem to be in harmony with the microorganisms. A situation can also be imagined where a species of microorganism may have in initial stages, created disease symptoms in an insect species or in closely related species. The resistant races or species tolerated this infection and gradually parasites became symbiotes.

Location of symbiotes

The symbiotes can be housed in various tissues (Fig. 24-1) in different insects. They enter a cell where they displace almost whole of the cytoplasm. Usually only one type of microorganisms (*monosymbiosis*) are associated with a species but in not so rare cases two or more different kinds of symbiotes are housed in a mycetome leading to *plurisymbiosis*. The different symbiotes are lodged in special cells or different regions of the same mycetome or in different mycetomes. In *Lyctus linearis* the two different species of bacteria are housed separately in cortical and medullary regions of the mycetome. In some species of Membracidae as many as six different types of symbiotes are found in different predetermined areas of the mycetome. In fulgorids different symbiotes are lodged in separate mycetomes. During transmission from one generation to another, a complicated mechanism operates in females to ensure that each progeny is infected with all types of symbiotes which enter the egg by different routes and are guided to their characteristic sites in mycetomal region.

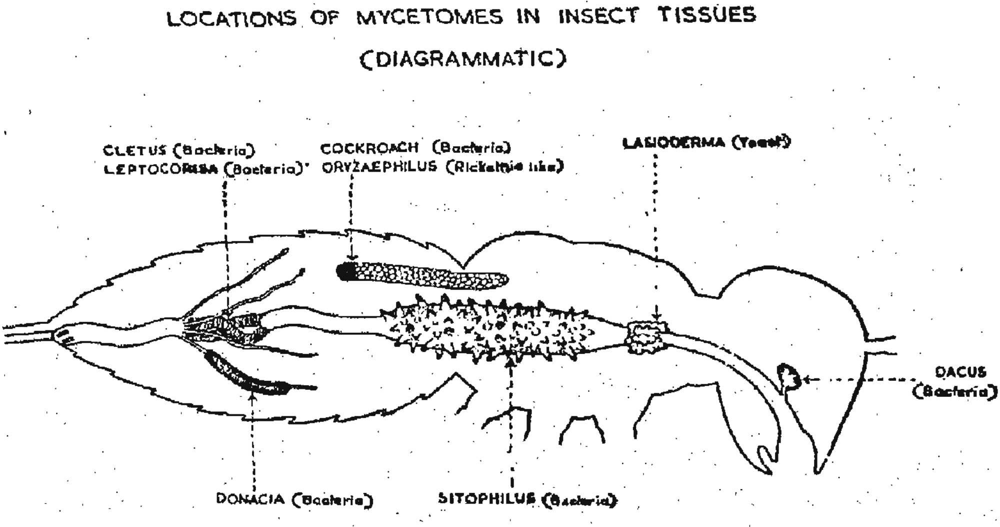

Fig. 24-1. Diagrammatic representation of the location of mycetomes in insects. The type of microbes are given in parenthesis.

In *Lasioderma serricorne* and *Stegobium paniceum* symbiotes are yeast like microorganisms and are located intracellularly in the mycetomes formed by evagination of epithelial cells at the junction of fore and midgut. In *Oryzaephilus surinamensis* and cockroach the mycetomes are attached to the fat bodies and have no connection with the alimentary canal. *Cletus* and *Leptocorisa* have bacteria filled caeca in the posterior part of midgut. One of the Malpighian tubes is modified to form a mycetome in the beetle, *Donacia*.

Transmission of symbiotes

Owing probably to the beneficial nature of endosymbiotic relationship, hosts have evolved several methods by which the continuity of this association is maintained from one generation to another. The symbiotes either enter the egg during early oogenesis or are placed in a capsule specially formed by the female for the young nymphs to 'drink' symbiote-filled liquid, or are smeared over the egg shell at the time of oviposition. We shall see the mode of transmission in a few typical examples.

Coptosoma scutellatum—This is a plant sap sucking heteropteran with mycetome in the lower part of the midgut. The midgut of female has a special apparatus which forms capsules filled with fluid containing bacteria. These capsules are deposited amongst the egg mass. When nymphs hatch they suck the contents of the capsule and thus symbiotes gain entry into individuals of the new generation.

Lasioderma and *Stegobium*—In females special transmission device exists. This consists of symbiote-filled transmission organs located at or near the opening of the common oviduct. As the egg is deposited, the transmission organs contract and smear the shell with a secretion containing yeast-like symbiotes. When the larva hatches, it eats up most of the shell and thus acquires infection of the symbiotes which upon reaching the midgut, penetrate the epithelial cells at the junction of the fore and midgut and form the typical mycetome.

Sitophilus oryzae—In ovarioles, the symbiotes enter the developing egg during early oogenesis. In larval stage the mycetomes are not attached to the gut but during pupation the cells containing symbiotes in larva fuse with the midgut and produce bacteria-filled caeca. During late larval stage, the symbiotes are liberated in the haemolymph which carries them to the rudimentary ovaries into which the bacteria enter.

Artificial elimination of symbiotes

The functions of symbiotes can be demonstrated easily if these are removed artificially in such a manner that the host is not adversely affected by the treatment that produces normal aposymbiotic progeny. A comparative study of the symbiotic and aposymbiotic populations of an insect species can then give an idea of the possible role and purpose of the symbiotic relationship. Several methods of eliminating symbiotes have been tried with varying degrees of success by different workers investigating physiology of symbiosis in insects. The choice of the method depends upon the mode of transmission of symbiotes. The commonly used methods are given below :

Sterilization of egg-surface: insects like *Lasioderma* and *Stegobium* symbiotes are smeared on the outer surface of egg shell which is eaten by the hatching larvae. If eggs are treated by a sterilizing fluid *e.g.*, 5% chloramine in 70% alcohol, the symbiotes are killed resulting in complete elimination of symbiotes in the new generation.

Centrifugation: When eggs of *Pediculus* are subjected to a high centrifugal force, the symbiotes in the yolk are displaced from their normal position and disintegrate. This method has not proved successful in other insects.

Thermal treatment: The symbiotes of some insects (*Oryzaephilus surinamensis, Sitophilus granarius*) are reported to be thermosusceptible. When these insects are held at temperatures of 30-35°C the microorganisms in larvae and adults show disintegration. Some workers have expressed doubts about the effectiveness of this method which at times fails to make insects completely aposymbiotic.

High oxygen tension: Termites containing protozoa when held under high oxygen tension lose their symbiotic fauna. This method however, has not been successful for the symbiotes located intracytoplasmically.

Microsurgery: This is a very difficult procedure requiring high precision instruments for removing mycetome from insect body by microsurgery. It was successfully applied in adult louse in which mycetomes are attached to the dorsal fat bodies.

Chemotherapy: Several types of bacteriostatic and bactericidal drugs are available commercially. These include different antibiotics (tetramycin, penicillin, chloromycetin, aureomycin *etc.*,), sulpha compounds and others. These chemicals can either be injected or orally administered. Their efficacy varies with different insects. In some, chemotherapy has resulted in complete or partial elimination of symbiotes while in others it failed to give any result as in *Sitophilus oryzae*.

Physiology of symbiotes

It is obvious that symbiotes derive benefit from the host which provides them with food and favourable environment without probably forcing the microorganisms to undergo degenerative adaptations. However, symbiotes are adversely affected if their environment (internal conditions of the host) is changed by factors like ageing of the host or malnutrition. The advantages to the host due to symbiotic relationship have been analysed in only a few insect species which could be made aposymbiotic.

Synthesis of vitamin and other nutrients by the symbiotes

Lasioderma serricorne and *Stegobium paniceum* contain yeastlike symbiotes. The transmission to subsequent generations is accomplished by smearing of symbiotes on the outer surface of the eggs during its passage through the common oviduct. If eggs are treated with 5% chloramine in 70% alcohol, the symbiotes are eliminated completely. The larvae which hatch from these eggs, do not contain any symbiotes and give rise to an aposymbiotic population. When the normal and aposymbiotic larvae of *Lasioderma* and *Stegobium* are grown on artificial diets lacking in one of the nutrients, the reactions are very different in the two sets of insect populations. The normal larvae of *Lasioderma* grow relatively well in diets which lack in riboflavin, inositol, choline chloride, nicotinic acid, pantothenic acid or folic acid (Fig. 24-2) but the aposymbiotic or sterilized larvae entirely failed to grow in the

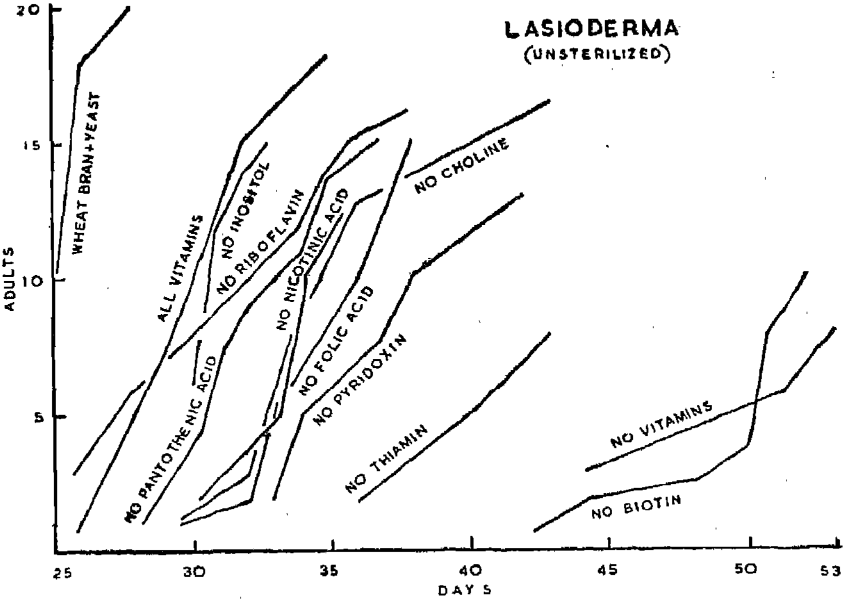

Fig. 24-2 Growth of unsterilized larvae of *L. serricorne* on artificial diets.

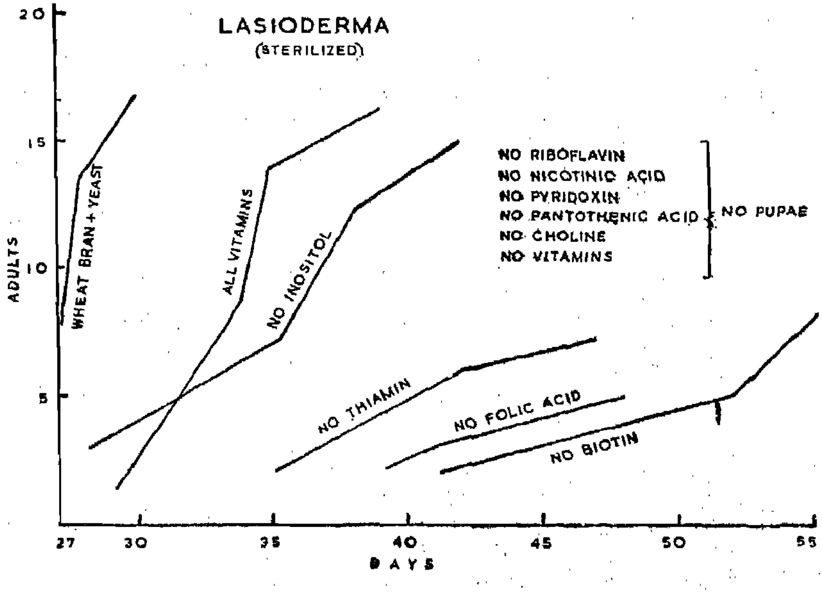

Fig. 24-3 Growth of sterilized larvae of *L. serricorne* on artificial diets.

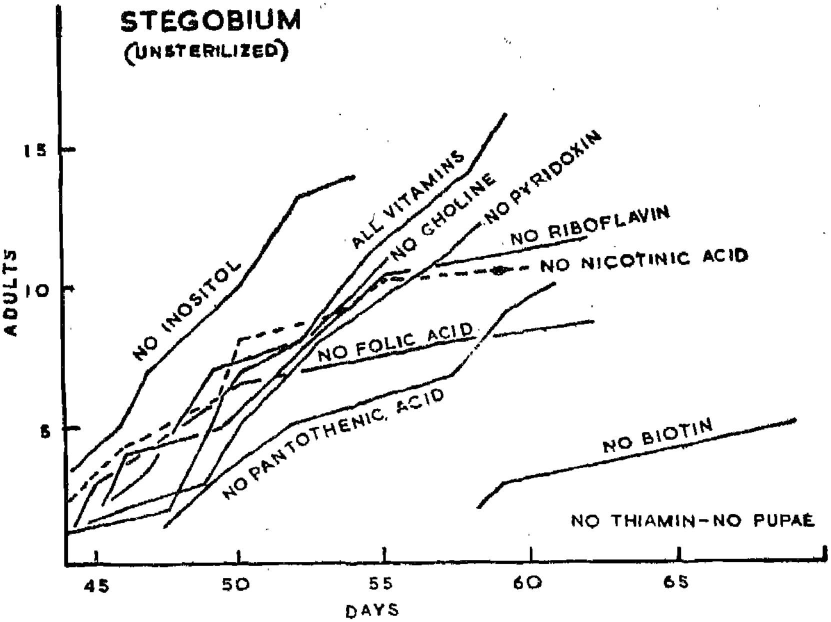

Fig. 24-4. Growth of unsterilized larvae of *S. paniceum* on artificial diets.

absence of riboflavin, nicotinic acid, pyridoxine, pantothenic acid or choline chloride (Fig. 24-3) showing that the symbiotes of *Lasioderma* supply these important vitamins to the host.

Similarly, normal *Stegobium* (Fig. 24-4) can tolerate dietary deficiency of pyridoxine, riboflavin, nicotinic acid, folic acid or pantothenic acid, with the help of symbiotes. The sterilized larvae deprived of the flora become susceptible to vitamin deficiencies (Fig. 24-5).

Besides vitamins the symbiotes of *Lasioderma* can also synthesize sterol for the host. Several essential amino acids are supplied to the host by the symbiotes in *Stegobium*. The aposymbiotic insects failed to develop in the absence of almost any one of the 10 essential amino acids. The normal larvae however easily overcome the deficiency of these substances (See Table on page 248).

In insects where aposymbiotic individuals cannot be obtained *in vitro* synthesis of nutrients by cultured symbiotes have been demonstrated as in *Sitophilus oryzae*, *Cletus* and aphids.

Vitamin C is essential for most of the insects especially the phytophagous forms. The symbiotes of the cockroach, *Leucophaea maderae* can synthesize vitamin C *in vitro*. Recently Singh (1971) proved that the symbiotes of *Cletus signatus*

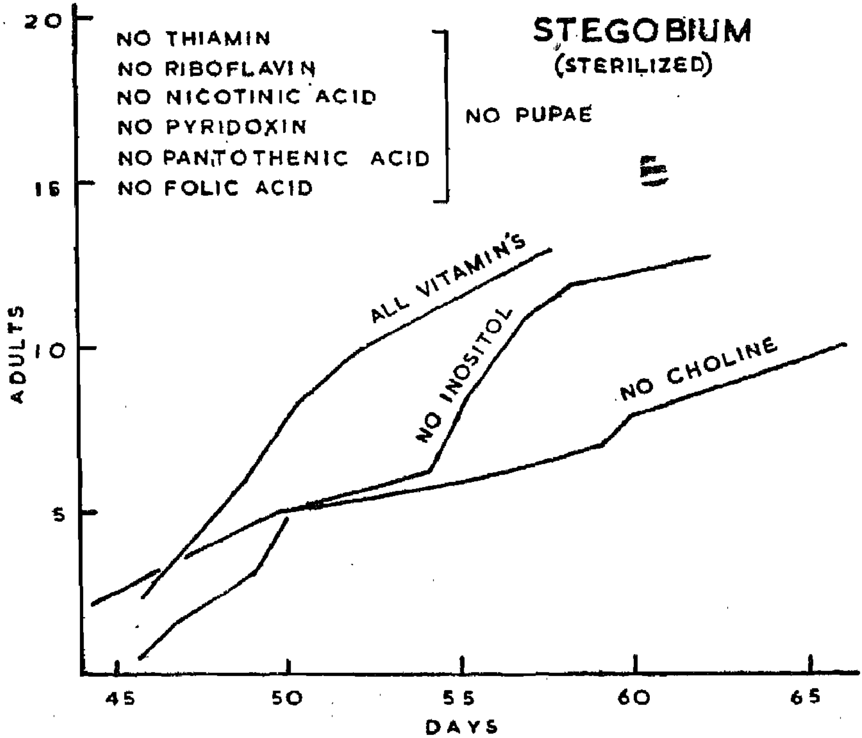

Fig. 24-5. Growth of sterilized larvae of *S. paniceum* on artificial diets.

(Coreidae) are capable of synthesizing vitamin C (ascorbic acid) from mannose *in vitro*. If kanamycin (an antibiotic) is added in the culture medium, the synthesis stops, indicating that it was due to bacterial activity. The kanamycin-treated adults however continue to show synthesis in their mycetomal tissues as the drug is quickly metabolished into harmless compound and the symbiotes are not adversely affected.

Effect of symbiotes on reproduction : In German cockroach the reproduction is adversely affected if symbiotes are removed. Wigglesworth (1952) suggests that the symbiotes in *Rhodnius* have some influence in the endocrine system of the insect. These functional attributes, however, lack clear cut experimental evidences.

An interesting role of symbiotes in sex determination in coccids of genus, *Stictococcus* has been reported by Buchner. The symbiotes invade ovary but infect only certain oocytes. Two types of eggs develop — one with symbiotes, giving rise

TABLE

GROWTH OF STEGOBIUM LARVAE (WITH AND WITHOUT SYMBIOTES) ON DIETS LACKING ONE OF THE AMINO ACIDS (FROM PANT *et. al.*, 1960).

Diets lacking in	*With symbiotes*		*Without symbiotes*	
	Number of adults out of 30 larvae	Developmental period in days	Number of adults out of 30 larvae	Developmental period in days
nonessential				
DL-Alanine	29	31—36	10	72—118
DL-Aspartic acid	24	35—57	18	43—118
DL-Cystine	26	32—57	17	52—118
L-Glutamic acid	24	33—57	16	52—118
Glycine	14	64—94	0	—
L-Hydroxyproline	27	33—57	17	35—72
L-Proline	28	31—47	23	40—118
DL-Serine	28	31—50	23	43—108
L-Tyrosine	29	33—50	18	43—108.
essential				
L-Arginine	2	51—78	0	—
L-Histidine	13	51—93	7	43—108
DL-Isoleucine	11	54—84	0	—
L-Leucine	9	54—88	0	—
L-Lysine	10	42—84	0	—
DL-Methionine	14	35—52	2	72—103
DL-Phenylalanine	18	35—86	0	—
DL-Threonine	7	57—78	0	—
L-Tryptophan	24	46—73	0	—
DL-Valine	13	42—75	0	—
No amino acids	26	38—57	—	—
Casein diet	27	27—37	29	28—40

to females and the other without them, giving rise to males. Cytological observations show that males are produced when one chromosome is lost. How does the presence of symbiotes prevent the abortion of a chromosome in female eggs, is not yet known.

Degradation of insecticides by symbiotic microorganisms: That the symbiotic bacteria in apple maggot can actively degrade toxic organophosphates and carbamates, was demonstrated by Mallory, Bousch and Matsmura (1967). The symbiotes, *Pseudomonas melophthora* produce a number of esterases in the culture medium, indicating that the microorganisms may be capable of hydrolysing natural esters for the host or help in detoxifying insecticides, thus providing protection to the host. Singh (1971) made a detailed study of the role of *Bacillus cereus*-like symbiotes of *Cletus signatus*. *In vitro* experiments he proved that the cultured symbiotes can degrade carbaryl, parathion and DDT quite rapidly. He could not make any *in vivo* investigations due to the failure in attempts to make *Cletus* aposymbiotic.

Applied significance of symbiosis research

The data collected during the last 25 years have established that the symbiotes of a number of insect species supply nutrients to their hosts. During this period insect nutrition as a subject by its own merit, became a favourite topic of research amongst economic entomologists who seek to utilize insect resistance in plants as a method of control. The relationship between the plant feeders and the plant crops cannot be interpreted to our advantage unless one takes into account the role played by the symbiotic microorganisms. Recent discoveries, that the function of the symbiotes extends to such regions as fixation of free nitrogen, sex-determination, degradation of insecticides *etc.*, call for an immediate step to augment researches on insect symbiosis which hitherto was regarded as a topic of interest only to a fundamentalist.

Works of Dang-Gabrani (1970), Singh (1971) and others have brought to the light the fact that symbiotes in general have probably not undergone parasitic type of degradation, in spite of the association being millions of years old. A comparative study of symbiotes in related genera can therefore form a basis for understanding the phylogenetic relationship of insect groups including physiological races.

Investigations on symbiosis can be of great interest not only to the physiologists, and taxonomists but also to ecologists who are concerned with the biotic associations of an animal species with plant and other animals.

The capacity of symbiote to metabolize insecticides and antibiotics offers very fruitful lines of investigations to toxicologists and physiologists in general. What mechanisms are involved in insects and symbiotes that even wide-spectrum antibiotics, fail to kill the bacteria in insect tissue? To what extent permeability of cell membrane of mycetome is responsible in imparting resistance to symbiotes? These are the few of several questions that are awaiting to be answered by the researchers.

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25

INTRACELLULAR SYMBIOTES AND THEIR FUNCTIONS IN SITOPHILUS ORYZAE L.

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Several types of microorganisms are known to inhabit insect tissues intracellularly. In most of the cases the mode of their association suggests that these are symbiotic in nature. Only in a few cases experimental evidence is available to prove the true nature of symbiosis between the microorganisms and their hosts. The organs which harbour symbiotes are called *mycetomes*. Although insects are not the only animals to live in association with microbes, their small size, wide degree of adaptation and distribution have made them specially suitable for disseminating and harbouring microbes of different types which could be bacteria, yeast, fungi or rickettsia-like organisms.

The consequences of microbial association in insects give rise to implications which are of interest to an economic entomologist. The vectorial capacity of insects for diseases in plants and animals including man has such a wide application that insect microbiology has become a distinct subject by its own merit. The amount of information available in the world literature now fully justifies insect-symbiosis as a subject distinct from insect pathology in which the association is detrimental to the host. The new domain of endo-symbiosis is full of promise where a biologist is interested to know, besides other things, how the partnership influences the life of an insect, *e.g., Sitophilus oryzae.*

Location of symbiotes: *S. oryzae* adult contains intracellular bacteria (*Bacillus circulans*-like microorganisms) located in the caeca of anterior midgut. These are lodged in the cells of distal part of the caeca which are histologically similar to the midgut and show epithelial cells and a narrow lumen. The mycetocytes (cells containing the symbiotes) are not in communication with the caecal lumen (Fig. 25-1). The caeca have three distinct cellular areas *viz.,* replacing cells, mycetocytes and midgut cells. In larva the mycetomes are not connected with the gut and lie in close proximation of midgut. During pupal stage these get attached to the gut and by the time adult characters are fully formed the caeca get infected with the bacteria.

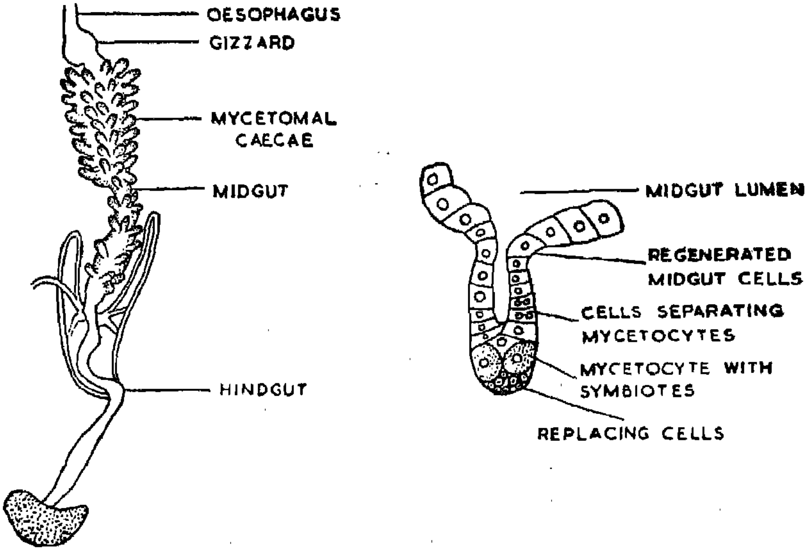


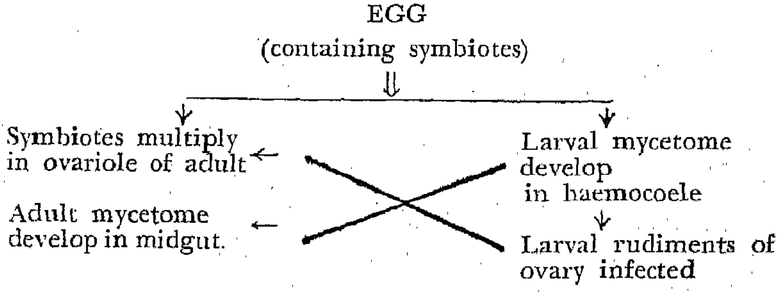
Fig. 25-1.

A. Alimentary canal of *S.oryzae* showing mycetomal caeca.

B. Internal structure of caecum showing mycetocytes.

Transmission of symbiotes: The symbiotes are inherited by each progeny. During oogenesis some of the bacteria leave the mycetome and go to infect the germarium where they lie scattered between the developing oocytes and nutritive cells. The infection probably takes place during second larval instar when the rudiments of ovary get infected by the bacteria. By the time the larva pupates, the developing ovarioles show heavy multiplication of the symbiotes.

A SCHEME OF TRANSMISSION OF SYMBIOTES IN
Sitophilus oryzae


EGG
(containing symbiotes)
Symbiotes multiply in ovariole of adult
Adult mycetome develop in midgut.
Larval mycetome develop in haemocoele
Larval rudiments of ovary infected

Artificial elimination of symbiotes: direct and reliable method for studying the functions of symbiotes is to remove artificially the microflora from the insect without adversely affecting the host. In insects where the symbiotes are transmitted externally through egg shell as in *Lasioderma* and *Stegobium* or by placing bacterial capsule among egg mass as in *Coptosoma,* the hosts can be made aposymbiotic without much difficulty. *Sitophilus* symbiotes are present inside the egg before the latter is laid. All methods including the use of antibiotics, high temperature, gamma radiation or centrifugation of eggs failed to eliminate the symbiotes.

The cultured symbiotes however proved susceptible to at least a few antibiotics like aureomycin and tetracyclin. When drugs are fed to the insect, these fail to kill the symbiotes. It appears that either the antibiotic is unable to reach the mycetome due to the selective permeability of cell membranes in mycetome or there exists a mechanism in insect body which metabolizes the antibiotics into ineffective compounds.

Vitamin synthesis by symbiotes: For insects a dietary source of vitamin B-complex is essential for growth and development. Out of 10 vitamins of B-complex nicotinic acid, pantothenic acid, riboflavin, pyridoxin, biotin and thiamin are considered very vital for insects which are not known to contain symbiotic *microorganisms.* Exceptions to this generalized rule are *Lasioderma, Stegobium* (Pant and Fraenkel, 1954), cockroach and *Rhodnius* (Wigglesworth, 1952) where the symbiotes synthesize these growth factors for the hosts.

That the symbiotes supply vitamins of B-complex is further supported by the fact that the cultivated symbiotes (which have been identified as very similar to *Bacillus circulans*) have been demonstrated to synthesize nicotinic acid, riboflavin and pantothenic acid *in vitro* (Dang-Gabrani, 1970).

Eixation of of nitrogen by symbiotes: Symbiotic microorganisms in the roots of certain plants have long been known to fix free atmospheric nitrogen. Whether such a phenomenon is attributable to the symbiotes of insects, is an interesting yet debatable issue. It is argued that since mycetomes invariably have a very rich supply of tracheae it is likely that symbiotes may be playing an important role in the nitrogen metabolism of the host. The symbiotes isolated from aphids, cicada and some heteropterous insects, can synthesize amino acids in culture. Certain wood-borers derive appreciable quantities of nitrogen with the help of their symbiotic flora. Amino acid synthesis has been demonstrated by the symbiotes of apple maggot, *Rhagoletis pomonella.*

When symbiotes of *S. oryzae* are grown on a nitrogen free liquid medium containing dipotassium monohydrogen phosphate 1.0 gm, magnesium sulphate 0.5 gm, ferrus sulphate 0.1 gm, calcium chloride 1.0 gm, sodium chloride 0.5 gm, sodium molybdate 0.005 gm and sucrose 20 gm dissolved in one litre of distilled water, the medium shows presence of nitrogen which is obviously fixed from air by the symbiotes. The amount of nitrogen fixed, depends upon the source of symbiotes. Isolates from the mycetome, fix 0.25 mgm while those from ovary and eggs, only 0.014 and 0.07 mgm respectively. These amounts are however much less as compared to 2-10 mgm of nitrogen fixed by *Azotobacter,* inhabiting root nodules. The symbiotes of *Sitophilus* are very similar to *B. circulans,* a free living bacteria which can fix small quantities of nitrogen. The role of symbiotes *in vivo* can be confirmed only if *Sitophilus* can be made aposymbiotic. This, unfortunately has not so far been achieved owing to the apparent resistance of symbiotes to several antibiotics and thermal procedures already discussed earlier.

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26

MODE OF ACTION OF ORGANOPHOSPHATES AND CARBAMATES

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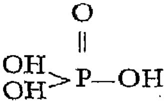
The discovery of organophosphates dates back to second World war when the compounds like sarin, tabun and soman were used as nerve gases. The use of these compounds in agriculture as pest-controlling chemicals has largely been due to the work of Gerhard Schrader of Bayer, Germany. Since then, the largest number of insecticides used, have been from this group and as early as 1959 more than 50,000 chemicals are estimated to have been synthesized with the addition of new chemicals each month. The carbamate compounds originated after the isolation of physostigmine or eserine in 1864 and its structural identification in 1925. Some of the carbamate compounds like sevin or carbaryl are very commonly used as insecticides these days.

Organophosphates are the esters of alcohol with a phosphorus acid or anhydrides of phosphorus acid with other acids. On the other hand, carbamates are the esters of carbamic acid. Their general formulae can be represented as given here.

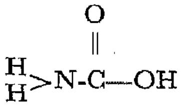
Organophosphate and carbamate compounds are perhaps the only groups of insecticides whose mode of action has been known with any certainty and a great deal is understood at the molecular as well as cellular levels on their interaction with the body systems. Such studies have not only resulted in far reaching understanding of the biochemistry of nervous

Acids



$$\genfrac{}{}{0pt}{}{OH}{OH}{>}\overset{\overset{O}{\|}}{P}-OH$$

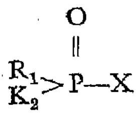
(Phosphoric acid)



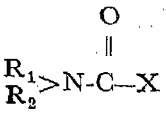
$$\genfrac{}{}{0pt}{}{H}{H}{>}N\text{-}\overset{\overset{O}{\|}}{C}-OH$$

(Carbamic acid)

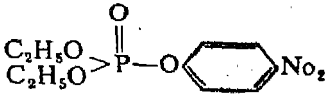
General Formula



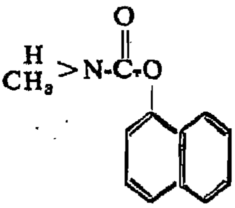
$$\genfrac{}{}{0pt}{}{R_1}{K_2}{>}\overset{\overset{O}{\|}}{P}-X$$



$$\genfrac{}{}{0pt}{}{R_1}{R_2}{>}N\text{-}\overset{\overset{O}{\|}}{C}-X$$

Example 

(Paraoxan)



(Sevin)

where R_1 and R_2 are alkyl groups and X represents a diversified form of structures containing OH.

system but also provided certain essential structural prerequisites in these compounds necessary for insecticidal activity. Informations regarding the action and metabolic degradations of these compounds in relation to their chemical make up in insect body, have opened the door of possibilities to synthesize the chemicals of desirable specificity and to avoid the problems of insecticidal resistance to insects and toxicity hazards to mammals.

It is now almost established that both organophosphates and carbamates are toxic because of their interference in the normal synaptic transmission of the nerve impulses. The physiology of nerve impulse transmission at synapses in insects is similar to that of parasympathetic nervous system of vertebrates involving the role of cholinergic components *viz.*, acetylcholine (ACh), acetyl cholinesterase (AChE) and choline-acetylase (ChA). As soon as an electrical impulse propagated along an axon reaches the presynaptic ending, it causes release of a transmitter substance, the ACh which is stored in pockets at the end of the axon. This diffuses across the synaptic cleft of about 500 A° width to postsynaptic fibres where it combines with the receptor site to produce an action potential, perhaps by altering the Na^+ permeability at that site. After an impulse has passed, the receptor should be brought to resting condition in order to maintain the sensitivity of the synapse. This is accomplished by removing ACh, the saptial temporal link by the enzyme AChE present on the presynaptic site, which hydrolyzes it into choline and acetic acid; the latter two can resynthesize ACh in presence of co-enzyme A and ChA (Fig. 26-1).

Toxic phosphoric and carbamic acid esters after penetrating inside the body of both vertebrates and invertebrates reach the cholinergic site and inhibit AChE by blocking its active sites which are responsible for the hydrolysis of the natural substrate ACh. The AChE inhibition thus leads to an accumulation of ACh at nerve endings, which causes dis-

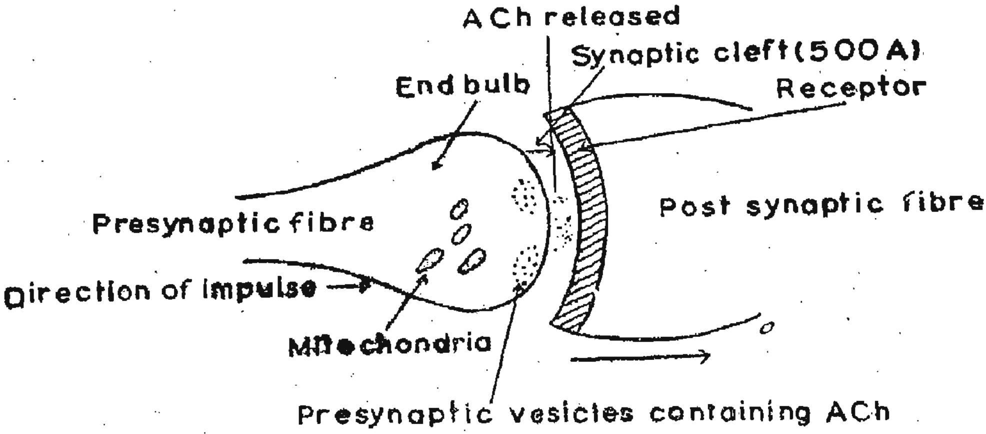


Fig. 26-1. Nerve impulse transmission at synapse in insects.

ruption of nervous activity resulting in excitation, paralysis and finally death. Out of numerous aspects of investigations in support of this mode of action of organophosphates and carbamates, the four most convincing experimental evidences are as follows: (i) The toxicity from these poisons is due to the nervous impairment, as evidenced by the developing symptoms including convulsion and excessive parasympathetic activity such as lachrymation, salivation, myosis *etc.*, in mammals, and excitability, tremor and paralysis in insects. (ii) The application of these compounds inside the body causes an increase in the level of ACh and a profound decline in that of AChE. Further, there exists a good correlation among the dose applied, per cent AChE inhibited and the mortality. (iii) Information on the essence of 'chemical structures' suited to influence the inhibitory potency of organophosphates and carbamates reveal that in general, very good inhibitors of this enzyme are very toxic and the poor inhibitors are poorly toxic to animals. (iv) No other vital enzyme has so far been either shown to be inhibited or its inhibition accompanied with toxicity.

The above information has convinced the majority of workers that the ChE inhibition is the biochemical lesion of organophosphates and carbamates in animals. In mammals the physiological consequences of the lesion have been completely traced out but no such information is available in insects and the immediate cause of death is not yet completely understood.

In general, cholinesterase (ChE) may be regarded as a family of enzymes which differs greatly from various animal sources with regard to substrate specificity and concentration pattern and sensitivity to inhibition. The various activities within this family may be divided into two groups: (i) *acetyl* *cholinesterase* (AChE) also known as true ChE or specific ChE which is distributed in grey matter of central nervous system, synaptic ganglia, motor end-plates and erythrocytes; this hydrolyzes ACh at a higher rate than butyrylcholine and propionylcholine and is inhibited by excess of ACh and (ii) *cholinesterase*, also known as pseudo-ChE or nonspecific ChE, which is found in white matter of central nervous system, liver pancreas, intestinal mucosa and plasma; it hydrolozes butyrylcholine and propionylcholine at higher rate than ACh and shows no substrate inhibition at excess of ACh. These two types of esterases can be differentiated by their substrate preference and use of selective inhibitors. Only the AChE is the principal esterase of animal central nervous system. It is this enzyme which is the target of organophosphates and carbamates. No biologically important functions are ascribed to pseudo-ChE and its activities can be completely inhibited without any harmful consequence.

The active centres of the AChE surface consist of two subsites. (1) An anionic site containing a nitrogen attracting group, *e.g.*, a negatively charged oxygen atom which strongly attracts the positively charged nitrogen of ACh (Fig. 26-2). This site facilitates enzymic activity by attracting, binding and orientating cationic substrates by virtue of Coulombic attraction and is largely responsible for the specificity of substrate. (2) The esteratic site contains a nucleophilic (basic) and an electrophilic (acidic) subsites, both providing the attracting force for the carbonyl group of ACh in which carbon is electrophilic and oxygen, nucleophilic. Esteratic site is concerned with the actual hydrolytic process and for this both the subsites represented by H—G are essential (Fig. 26-2).

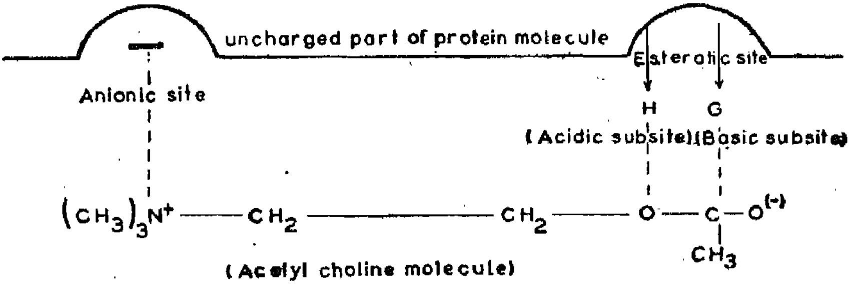
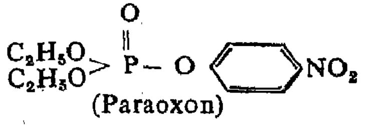
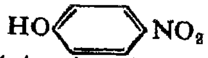
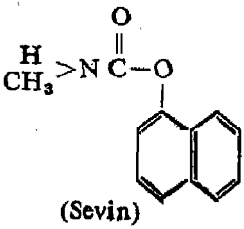
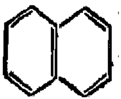

uncharged part of protein molecule
Anionic site
Esteratic site
H
G
(Acidic subsite)(Basic subsite)
(CH3)3N+ — CH2 — CH2 — O — C — O(-)
CH3
(Acetyl choline molecule)

Fig. 26-2. Active sites in acetyl cholinesterase and attachment of acetylcholine molecule on its surface.

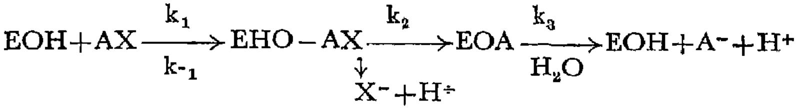
The mechanism of ACh hydrolysis is similar to nonenzymatic OH^- hydrolysis, the OH group at esteratic site comes from perhaps the imidazole group of histidine and it is a 3-step process. In first step, the formation of a reversible complex between ACh and ChE takes place. In second step, the nucleophilic reaction of OH⁻ group of enzyme occurs with electrophilic carbon atom and the transfer of a proton from the acid group of enzyme to the nucleophilic part of the substrate (oxygen of carbonyl group) leads to the acetylation of the enzyme and liberation of choline. In the third step, the acetylated enzyme in presence of H_2O or other nucleophilic agents like OH^-, NH_2OH *etc.*, is cleaved into enzyme and acetic acid.

The chemical reaction between ChE and organophosphates or carbamates is similar to that between ChE and ACh, *i.e.*, the basic group of enzyme makes a nucleophilic attack upon the positive phosphorus atom of organophosphates or carbon atom of carbamates leading to the split of these esters by an electronic and protonic shift, but unlike ACh where both the fragments of hydrolysis *i.e.*, choline and acetic acid are separated from the enzyme, the P and C atoms of the inhibitors remain firmly bound to the esteratic site of the enzyme forming relatively more stable compound in the form of phosphorylated or carbamylated enzyme. The modern concept of molecular interaction between ChE and organophosphates has been put forward by Main and Iverson (1966) and that between ChE and carbamates by Wilson *et al.*, (1960).

In order to understand the reaction of substrate or inhibitors with enzyme in generalized manner, their molecules can be sought to have two groups. The first group is attached with the enzyme in the process of acetylation, phosphorylation or carbamylation and the second is the leaving group which does not participate in the above process. The leaving group of ACh, paraoxon and sevin are shown below:

Compound	Leaving group
I. $(CH_3)_3N^+CH_2CH_2OCOCH_3$ (Acetyl choline)	$(CH_3)_3N^+CH_2CH_2OH$ (Choline)
II.  (Paraoxon)	 (p'—nitrophenol)
III.  (Sevin)	 (1—naphthol)

If these leaving groups are denoted by X and the remaining part of these molecules *viz.*, acetyl group of ACh, diethyl phosphoryl group of paraoxon and methyl carbamyl group of sevin by A, the generalized reaction of these compounds with enzyme can be expressed as follows:



$$\mathrm{EOH+AX}\xrightarrow[k_{-1}]{k_1}\underset{\substack{\downarrow\\ X^-+H^+}}{\mathrm{EHO-AX}}\xrightarrow{k_2}\mathrm{EOA}\xrightarrow[H_2O]{k_3}\mathrm{EOH}+A^-+H^+$$

where EOH represents the enzyme; EOH-AX, the reversible complex; and EOA, the acetylated, phosphorylated or carbamylated enzyme. The k_1, k_{-1}, k_2 and k_3 indicate the reaction rates. The $\frac{k_{-1}}{k_1}$ is also called K_a representing affinity constant between enzyme and substrate or inhibitors. The difference in the mechanism of reaction of enzyme with substrate and the inhibitor is due to the different K_a, K_2 and k_3 values in each case.

For ACh, the k_2 and k_3 are very fast so that acetylation and hydrolysis are very rapid. The K_a or the affinity constant depends upon the structural similarity of the compound with ChE and since the natural body substrate ACh has a very high affinity with enzyme due to K_a being very small, the formation of the complex EOH.-AX is much favoured. A similar reaction of enzyme with organophosphates and carbamates does not lead to hydrolysis of these compounds due to the inhibition of ChE because of the least k_3 values resulting in accumulation of EOA in which group A of these inhibitors is attached to the esteratic site of ChE making it unable to react with ACh for hydrolysis of the latter.

Difference between organophosphate and carbamate inhibition

For reaction between ChE and organophosphate the values of k_1, k_{-1} and k_2 are such that under practical conditions, the complex EOH-AX never tends to accumulate. The k_2 is sufficiently fast *e.g.*, 30/min but the rate of hydrolysis (k_3) is absolutely low or nil. This causes EOA to accumulate and this is the only form which represents the inhibited enzyme. On the other hand with carbamates, the K_a is exceptionally low resulting into very quick complex formation. The much lower values of k_2 *e.g.*, 1/min permit EOH-AX to be normally present and is accumulated. Further, significantly smaller k_3, *e.g.*, 0.05/mm (although enough, when compared with that from organophosphate) results in the accumulation of EOA. Thus, under practical conditions there exists a small level of EOH-AX and a high level of EOA and both these forms contribute to the total experimentally determined carbamylated enzyme.

The EOH-AX accumulation and higher k_3 values in carba mylation make it different to phosphorylation in following sense:

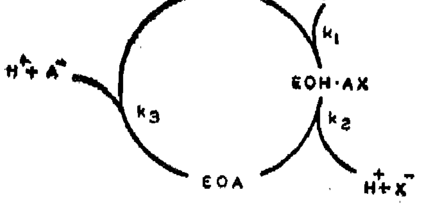
(a) The contribution due to EOH-AX in total carbamate inhibition depends to a greater extent on the concentration of substrate and its affinity with enzyme. If one adds a substrate of very high affinity, like ACh at high concentrations *e.g.*, 10^{-2}M in order to stop the carbamylation of ChE, the observed inhibition would be entirely due to EOA, because the entire complex EOH-AX gets reversed under these conditions. On the other hand if one uses lower concentrations of a low affinity substrate then in addition to EOA, much of the EOH-AX form will contribute to the observed inhibition. This type of sensitivity in inhibition by change in substrate concentration is not observed with organophosphates because of the absence of EOH-AX accumulation.

(b) If a carbamate compound is used as ChE inhibitor in higher concentration to be equal to K_a, then for the first few seconds more than half the enzyme molecules are in the form of EOH-AX without any appreciable formation of EOA. But the experimentally observed inhibition by using higher substrate concentrations, as is normally done to stop the further inhibition, would be zero for this period. This is because in carbamate inhibition the substrate molecules complete with the inhibitor for the enzyme surface and hence the inhibition with carbamates is competitive. In case of organophosphates on the other hand, the inhibition of ChE is non-competitive.

The rate of recovery of phosphorylated ChE is slow as compared to that of carbamylated enzyme. The normal activity of carbamate-inhibited enzyme is restored within few hours after the excess of carbamate has been removed, whereas the ChE inhibited by organophosphates returns to its normal activity after several days. The reason of this quick recovery of carbamylated ChE is that after the excess of inhibitor is removed either by dialysis, great dilution or by passing the solution through Sephadex (modified dextran), the enzyme gets recovered partly by decarbamylation or hydrolysis (k_3) and partly due to the reversal of EOH-AX.

(c) The terms *irreversible* and reversible inhibitor have long been used to differentiate the inhibition of ChE by organophosphates and carbamates respectively. The carbamates were earlier thought to inhibit ChE due to a competitive absorption of these compounds on the enzyme molecules, while OP-inhibition is due to an irreversible chemical reaction in which the enzyme is phosphorylated. It is now understood that only a part of the inhibited enzyme present in form of EOH-AX can be reversed. The restoration of ChE activity from carbamylated form is certainly not due to reversal of k_2 but due to the hydrolysis. Therefore, the carbamate inhibition may be said to be reversible from the point of view of enzyme, but not with inhibitor which is degraded in the process.

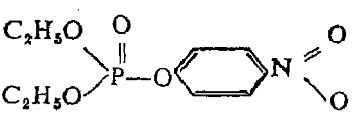
(d) In spite of the fact that carbamates behave like reversible inhibitors in that the ChE can be recovered by dialysis and that the concentration of substrate used for estimation of inhibition has a great impact on the results obtained, their reaction with ChE differs from those of reversible inhibitors in being progressive with time. Nevertheless the time curves for carbamates differ from those of phosphates in two respects. First, the time dependence in carbamate inhibition is exhibited only by EOA and not by EOH-AX, and second, the total inhibition of ChE never takes place in practice with carbamates as occurs with organophosphates. This difference is due to two phenomena. First, soon after one adds the substrate during the assay the formation of EOH-AX is blocked and the carbamylated enzyme (EOA) begins to recover through hydrolysis (k_3) reaction. Second, even in the presence of carbamate concentration 100 times greater than K_a, the total carbamylation of enzyme is never encountered due to the continuous availability of fresh enzyme after decarbamylation and continuous degradation of carbamates in the process as shown below:



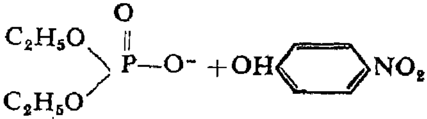
Majority of organophosphates are such that these attack only esteratic site of the ChE except a few *e.g.*, soman, whereas carbamates orientate both the anionic and esteratic sites except dialkyl carbamyl fluorides and a few others. The reactivity of these compounds to ChE depends mainly on two factors:

(a) the groups attached to P or C should induce an electrophilic character to these atoms by increasing their positivity, and (b) these compounds should have a configuration similar to ACh, the natural substrate of ChE. In case of organophosphates, the electronic factors are more important while for carbamates, the steric factors are of major significance.

Those organophosphorus molecules in which the electropositivity of P atom is high are always good inhibitors of ChE. Thus, phosphates [$(RO)_2P(O)OX$] can inhibit ChE activity while phosphorothionates [$(RO)_2P(S)OX$] cannot. This is due to the higher electronegativity of oxygen (3.5) than sulphur (2.5) and so the former possesses greater power of electron withdrawal from phosphorus rendering it more positive than what sulphur can do. Similarly, the hydrolytic products of organophosphates are inactive because of the negative charge on oxygen of X position and so it is repellent towards the electrons of phosphorus.



Paraoxon
(potent ChE inhibitor)

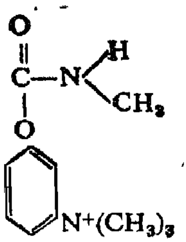


Hydrolysis products of paraoxon
(no inhibition property).

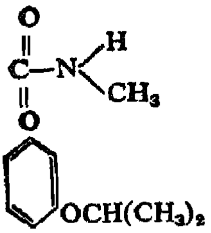
The stability of inhibitors depends similarly on the electron releasing nature of the groups attached to P atom. The most stable would be the compounds having least electron releasing groups because these do not decrease the positivity of P atom. The electron releasing character of the groups generally present in organophosphate molecules, can be arranged as $F < OR < SR < NR_2$. The stability of compounds increases with the increase in C chain of R_1 and R_2 from 1 to 3 followed by again a decrease due to some other reason.

On the other hand, the role of electronic factors for carbamate inhibition becomes clear from the investigations of Casida and associate (1960) who found that although the electrophilic substituents upon X accelerated the reaction for both organophosphates and carbamates but these accelerated the inhibition reaction for organophosphates, and slowed it in case of carbamates. It is now known that k_2 step is undoubtedly electrophilic, the k_1 step is very probably nucleophilic and the retardation in inhibition reaction with carbamates takes place due to the relatively greater contribution of K_a than k_2.

Besides structural closeness to ACh, the carbamates should be unionized in order to possess insecticidal property. Neostigmine is very similar to ACh and is a very potent ChE inhibitor, but it is not insecticidal because, like ACh and other ionized or ionizable compounds, it cannot enter the nerve membrane. A similar but unionized compound on the other hand shows the modest insecticidal activity.



Neostigmine
(non insecticidal)



Baygon
(insecticidal compound)

Inhibition and toxicity relationship

Chadwick and Hill observed a direct relationship between the doses of DFP applied on American cockroaches and the per cent ChE inhibited. This curve was nearly parallel to that of concentration of DFP and consequent mortality. A similar correlation was also found between per cent ChE inhibited and symptoms exhibited in honey bees treated with parathion and TEPP. About 50 to 60 per cent ChE inhibition was shown to be necessary for developing hyperactive and knockdown symptoms respectively (Metcalf, 1955). On the other hand in vertebrates, the nerve conduction is not reported to be affected unless ChE inhibition amounts to about 90 per cent.

This anomaly was solved by Stegwee in 1952 by showing that *in vitro* treatment of parathion although did not inhibit the ChE in nervous system of cockroach, the leg muscles had lost some 50 per cent of ChE activity. Further during hyperactivity after injection with parathion in cockroach, ChE in the central nervous system was inhibited from 54-74 per cent while that in leg muscles from 88-90 per cent. This showed that hyperactivity might be caused due to peripheral nerves, myoneural junctions or sense organs. The correlations between toxicity, symptomology and *in vivo* inhibition of the brain ChE of houseflies, treated topically with 17 organophosphates and three carbamates have been shown by Mengle and Casida (1958). Later, numerous other workers confirmed that in insects ChE is largely inhibited by organophosphates within a few hours, but always recovered in the survivors up to 90 per cent or even more of the normal, within one day.

Comparatively little work has been done on such relationships with carbamates. Cockroaches injected with physostigmine yielded the inhibition of the nerve cord ChE and this appeared to be related with toxicity (Chadwick, 1963). A good correlation was found between anti ChE activity and mortality with 32 carbamates to thrips but not to houseflies because of the rapid metabolic degradation in latter insects. Casida *et al.*, (1960) showed that *p*-nitrophenyl isopropyl carbamates were good and anti-ChE but non-toxic. On the other hand for dimethyl carbamyl fluorides reverse case was observed.

Besides ChE, the other esterases in insects are aliphatic (AliE) and aromatic esterases (AroE). ChE and AliE are sensitive to organophosphates as well as carbamates at specific concentration ranges, while AroE are not affected by these compounds. The best substrate for AliE is methyl butyrate and that for AroE, nitrophenyl acetate. During the early part of ChE studies in insects, it was shown that unlike mammals the toxicity due to organophosphates was because of the inhibition of other esterases in insects. No hydrolysis of ACh could be demonstrated by the homogenates of *Tenebrio molitor* and *Tribolium castaneum*, while methyl butyrate and O-nitrophenyl acetate were found to be hydrolyzed by these homogenates. Further, this hydrolysis was shown to be inhibited by organophosphates like TEPP and parathion. This and similar other studies proved AliE inhibition to be crucial in insect toxicity. This discussion, however came to an end in 1960 after the work of Stegwee who clearly established that by using TOCP one can specifically inhibit all the AliE of housefly without any toxic symptoms and ill effects on the insect.

O'Brien (1967) treated houseflies with parathion, malathion, diazinon and Co-Ral and recorded the resulting inhibition in hydrolysis of ACh, phenyl acetate, tributyrin and methyl butyrate at various intervals after poisoning. The results obtained showed that in surviving flies, the ChE was invariably the least inhibited enzyme whereas in killed flies, ChE was usually the most inhibited enzyme being 75.99 per cent inactivated. The difference between enzyme levels of killed and surviving flies, at any time was also greatest for ChE. It was concluded on this basis that the death is associated with ChE inhibition. Fortunately for carbamate compounds, no such problem has arisen.

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27

PHYSIOLOGICAL EFFECTS OF IRRADIATION

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SINCE the discovery of X-rays by Roentgen in 1895 and that of natural radioactivity by Becquerel (1896) we have come a long way in understanding the mechanism of radiation action and the various physiological and biochemical changes which radiations bring about in a living organism. The term radiation indicates a physical phenomenon in which energy travels through space which may even be empty. Ionizing radiations are so called because these bring about ionization in matter through which these pass.

Mechanism of radiation action

As to how irradiation brings about changes in an organism leading to its death, there are two views, namely the direct action theory and the indirect action theory. However, it must be borne in mind that these views are not contradictory but only supplementary to each other.

Direct action theory : As per this theory it is considered that when a swiftly moving charged particle passes through or near a complex biological material, the biological function of the complex can be altered or even destroyed. There is every probability that ionization may occur in a very vital molecule such as an enzyme or nucleic acid. Radiobiologists consider such a possibility of a direct hit on a biological target as the direct action. Crowther in 1924 postulated that the death of a cell results from a hit on a sensitive spot. The sensitive spot may be a DNA molecule or any other vital molecule of a cell. Crowther's *hit* theory was duly amended by the *target* theory which postulates that the incidence of a 'hit' is related to the size of the sensitive structure or molecule; the fewer the 'hits' required to inactivate a cell, the larger the structure must be.

Indirect action theory: The ionization being not a selective process, any atom or molecule in the path of a radiation may get ionized. In such a condition the predominant constituent of a cell stands the best chance of being ionized. The chief constituent of living material in its functional state is water. The water content not only differs from organism to organism but also from one tissue to another. It may constitute 70% to 96% of the weight of an organism. Thus water becomes the most probable material in which ionization can occur by radiation. The resulting chemical products may react with biologically important organic molecules and thus be indirectly responsible for an absorbable effect.

The concept of indirect action by radiations had lead to both extensive and intensive studies on radiation chemistry of water. The primary radiochemical action of water is believed to consist in splitting the water molecule into H atoms and OH radicals through ionization and excitation of water molecules. These two primary products are chemically very reactive and can act as oxidizing and reducing agents and even can break C—C bonds. Being highly reactive these are short lived (10^{-5} sec.) and have a diffusion distance of 30A° on the average. Although H atoms and OH radicals are believed to be the poisoning products of the interaction of radiation with water, secondary products such as hydrogen peroxide and HO_2 (perhydroxy radical)resulting from the interaction of primary products may be of considerable importance. These secondary products are chemically stable and have a greater diffusion distance. Thus both primary as well as secondary products of water may bring about a number of oxidation-reduction reactions in a cell leading to its disruption.

According to the indirect action theory the biologically active material along with the medium constitutes the target, whereas the direct action theory confines the target to a particular molecule in which the primary event, ionization has to occur or through which or near which an ionizing particle has to pass through. In biological systems the effects of ionizing radiation differ quantitively when the radiation dose is changed. Small doses act by indirect action and large doses by both direct and indirect. When a biologically active material such as an enzyme is irradiated dry, any action of radiation is necessarily of direct type. On the other hand when the material is irradiated in its functional hydrated stage, the action can be either direct or indirect. Hutchinson and his co-workers studied the radiosensitivity of enzyme of yeast cells in wet and dry conditions and concluded that the enzymes in dry condition were radioresistant. The same enzymes in hydrated condition proved to be highly radiosensitive. It is generally known that dry seeds are more radioresistant than those which have taken up water and also the radiosensitivity of seedlings increases with increasing water content.

Effect of radiation on biological system

Effect at cellular level: As the cell is the fundamental unit of life in all biological systems a proper understanding of the various effects of radiation in a cell will lead to a better understanding as to how the various physiological changes are brought about in an irriadiated organism. Much discussion has been devoted to the problem of whether the site of the primary lesion resides in the nucleus or in the cytoplasm of the cell. As early as 1932 Zirkle showed that nucleated part of the spores of *Pteris longifolia* was much more radiosensitive to α-polonium particles than the non-nucleated part. Similar results were observed with eggs of *Habrobracon juglandis*.

To test whether the site of the primary lesion resides in the nucleus or in the cytoplasm of the cell, studies were carried by transplanting the irradiated nuclear part to non-irradiated cytoplasm and *vice versa* in amoeba. Amoeba with irradiated nucleus died within 3 days while that with irradiated cytoplasm survived 3 weeks or more. It appears that the irradiated cytoplasm actually relayed the ionization damage to the unirradiated nucleus. Somewhat different results were obtained by other workers while investigating the effects of irradiation on the nuclear and non-nuclear part of the eggs taken directly from the ovaries of frogs and salamanders which constitute a very radiosensitive vertebrate material. The nuclei, free of cytoplasm were found to be very radiosensitive. When isolated normal nucleus was placed in cytoplasm and the whole reconstituted structure was irradiated, the nucleus showed high radiosensitivity. Transferring the nucleus of an irradiated egg to the enucleated cytoplasm of a normal cell did not show any lesions but in a normal nucleus when transferred to irradiated cytoplasm the changes became apparent. This shows that the nucleus may not necessarily be the site of primary lesion. If, as little as 10^{-44} ml of cytoplasm from a ovarian egg irradiated several days previously was injected close to the nucleus into the cytoplasm of non-irradiated cell, all the symptoms of radio-lesions such as a rupture of the nucleolus, vacuolization and chromosome fragmentation appeared just 1 or 2 hours after injection.

The earliest biochemical changes following irradiation are characterised by an increase in the activity of several enzyme systems. Some workers proposed that for cell death the primary lesion is an alteration in the permeability of intracellular structure like mitochondria. At cellular level biochecal processes are kept under control due to distinctly separate localization of enzymes and substrates within the same cell. The release of enzymes immediately after irradiation is the main disturbing factor which causes radiation injury. De Durve of Belgium provided clear cut evidence of the participation of the lysosomes (in which many of the hydrolytic enzymes of the cell are concentrated) in cell lysis. The lysosomes are the "suicide bags" of the cell as these contain autocidal substance for the destruction of the cell. Normally, the proteolyic and nucleic acid-attacking enzyme are confined in the lysosomes and do no harm. But at some stage during death of the cell, these are released to destroy the macro-molecules. When the liver is rendered anorexic, the proteolytic enzymes in the lysosomes are activated by a drop in pH associated with anaerobic metabolism. The lysosomes break down and the released hydrolytic enzyme destroy the cell. Although there is no direct evidence that the lysosomes are broken down by radiation resulting in the release of hydrolytic enzymes leading to the death of cell, yet in complex biological system even such a distant probability cannot be ruled out.

Effect on higher animals: The ultimate effects of irradiation on the living cell may be greatly affected by the physiological state of the cell. Normal metabolic processes seem to be responsible for the development of various lesions from the molecular level to the anatomical level. Ancel and Vintemberger observed that hen's egg kept in refrigerator before and after irradiation did not show any lesions even three days after irradiation. However egg incubated for 3 days following irradiations, showed a number of lesions. Later in 1930 Vintemberger wrote that, "the duration of survival of an irradiated cell is inversely proportional to its activity after irradiation." Similarly, Lamarque and Gros. in 1953 observed that irradiated silkworm eggs kept at low temperature for six months did not show lesions. Hibernating mammals are less radiosensitive when irradiated and maintained in hibernation. On warming 2-4 weeks after irradiation, the animals show the same amount of radiosensitivity as that in non-hibernating mammals. Thus metabolism appears to have an important role in the development of a lesion.

The general effects of irradiations on the mammals show the following symptoms : (i) Anorexia or less utilization of oxygen, (ii) loss of water and electrolytes resulting from diarrhoea, (iii) vomiting or disturbances in capillary permeability, (iv) endocrine disturbances, (v) slowing down of DNA synthesis, (vi) an early increase in catabolic and anabolic processes in the bone marrow, spleen and haemocytes forming the cells, (vii) little change in general metabolism except during last days of life, (viii) carbohydrates, fats and proteins are absorbed in spite of precarious anatomical condition of the intestine and (ix) uncoupling of oxidative phosphorylation.

Effect on insects: It is well known that the insects are much more resistant to irradiation effects than mammals. The cells during division and differentiation are most sensitive to irradiation. In mammals, cells are actively being formed throughout the greater part of their life. In insects on the other hand formation and differentiation of cells occur mostly during embryonic development and in post embryonic stage the growth occurs primarily by the enlargement of cell volume without an increase in cell number. It is for this reason that the post embryonic stages, notably the adults are highly radioresistant. The most vulnerable stage in insect cycle is the egg stage which is highly radiosensitive and requires much smaller dose to cause radiation injury as compared to larva, pupa and adult. For example, in case of *Bracon hebetor* the lethal dose for embryo is 100 r, while the same for old pupae is 100,000 r. This phenomenon of sensitivity of cell to radiation has been summed up in what is known as "Bergonie-Tribondeae Law" which states that the sensitivity of cell to irradiation is directly proportional to their reproductive (multiplication) activity and inversely proportional to their degree of differentiation.

The insensitivity of adult insect to irradiation is due to the peculiar static quality of the adult insect cell. Even in adult insects, sensitivity of various tissues differs and the tissue in active division being the most sensitive. This being the case we find large differences between the sterilizing dose and the lethal does for insect. In the case of *Bracon hebetor* the sterilizing dose (5000 r) is 60 times lower than the lethal dose (300,000 r).

As in mammals, irradiation causes anorexia in insects. As early as 1956 Grosch observed that somehow radiations interfere in feeding and utilization of nutrients by an insect. Since then it has been confirmed in many insects. Wharton and Wharton (1959) studied the normal daily intake of male cockroaches irradiated with 10,000-20,000 r and found that their food intake decreases with increase in dose. Similarly in *Tribolium castaneum*, (Roger and Hilchey, 1960) and *Anthonomus grandis* (Bartlett *et al.*, 1968) the food intake decreased in irradiated individuals. Recently Rao (1970) studied the effects of irradiation at three different doses namely 1000, 2500 and 5000 r on *Schistocerca gregaria* in greater detail. It was observed that the food intake of the irradiated adults decreased with increase in dose and time. By the 10th day after irradiation the insects in 2500 and 5000 r dose treatments completely failed to feed. The weight gained by the irradiated insects for ten days period was significantly lower to that by non-irradiated ones.

Studies on the effects of X-rays on respiration of the eggs of *Melanoplus* showed that doses in the range of 300 to 900 r cause transient respiratory inhibition while in the range 1000 to 2000 r the effects were the same but they failed to hatch. However, according to other workers these eggs had stimulatory effect on respiration by 25000 r (up to 48%) with inhibition at higher doses (200,000 r). In *Chortophaga viridifasciata* X irradiation leads to inhibition of respiration.

In *Melanoplus differentialis* an unidentified oxidase is first rapidly depressed by irradiations, then recovered and finally stimulated. Both effects are greatest at the lowest dose (25000 r). Further, it was also observed that SH (sulfahydryl) groups were unaffected at first but greatly inhibited after 15 days. Ito and Tanaka (1963) observed that irradiation of *Bombyx mori* causes inhibition of the enzymes associated with midgut. However Akov (1966) failed to find any difference in the proteolytic activity of *Aedes* after irradiation.

Wharton (1961) studied the effect of irradiation on starved adult male cockroaches with 10,000 r. They observed in irradiated roaches (i) increased water consumption, (ii) lowering of faecal pH, (iii) increase in nitrogen and uric acid excretion and (iv) no change in inorganic phosphorus excretion. They were of the opinion that increased cellular permeability was responsible for the above effects and thus it may be the site of primary lesion. Rao and Mehrotra (unpublished data) observed no difference in the approximate digestibility (A.D.) of dry matter and carbohydrates between irradiated and unirradiated adults of *S. gregaria*. However, nitrogen digestibility was seriously affected by radiation leading to negative nitrogen balance in the treatment, except for the first 6 days of the experimental period, during which the gross efficiency of 5000 r treatments was significantly higher as compared to non-irradiated ones owing to greater maintenance requirements of the latter. No significant difference in either gross or net efficiency of food utilization to body matter was observed between irradiated and non-irradiated insects.

The work so far done in insects on the physiological effects of irradiation is comparatively little and work in this field will be highly rewarding.

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28

CHEMOSTERILANTS AND THEIR MODE OF ACTION

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POPULATION explosion seems to be the common characteristic of animals including man and insects. Whereas it is dangerous in the long run to allow the population of animals and man to grow unrestricted, it is almost fatal in our interest to permit explosion of insect population even for a short duration.

The shortcomings of chemical methods of insect control are too well known to be listed here. In order to circumvent some of their ill effects, use of chemosterilants, sex-attractants and resistant plant varieties have recently been added as new tools in the armoury of the scientists so that dependence on chemical control may be supplemented, much less replaced.

The term chemosterilant was coined by LaBrecque *et al.*, in 1960. The theoretical basis of the use of chemosterilants for eliminating sexually reproducing organisms was first outlined by Knipling (1955). His dream took shape when classical successes were attained by complete eradication of the screw worm fly from the island of Curacao (Baumhover *et al.*, 1955; Lindquist, 1955) and Florida and south eastern United States by the release of 3.5 billion sterile flies in 85,000 sq. miles over a period of seventeen months.

The success with the screw worm flies with gamma irradiation had the seeds of development of the chemical sterilants envisaged by Knipling as more practical and less costly method of insect control without the necessity of rearing and releasing the vast number of irradiated insects.

The history of the progress in this direction can be divided into three periods *viz.*, (i) 1940-50: use of chemical mutagens inducing sterility in insects, (ii) 1950-60: use of antimetabolites that induced sterility in females by inhibiting egg production and (iii) 1958 onwards: use and development of chemosterilants.

Prior to 1958 there was no reference to chemosterilization of insects. There were only a few papers relating to the mutational effects of mustard gas and related compounds (Auerbach, 1947; Auerbach and Robson, 1947). The sterilant effects were produced by alkylating agents (Bird, 1950), by folic acid antagonists such as aminopterin (Goldsmith and Frank, 1952) and by mitotic poison on the housefly (Mitlin *et al.*, 1957). An extensive screening programme for chemosterilants was initiated in 1960 by the USDA to find chemicals that would sterilize insects. Over 3000 chemicals have been tested so far.

Borkovec (1962) defined *chemosterilants* as chemical compounds which, when administered to the insect, would deprive it of the ability to reproduce. Crystal (1964) called them chemical substances that prevented production of viable progeny. Borkovec (1966) modified his definition by stating that these reduce or entirely eliminate the reproductive capacity of an animal when administered. LaChance *et al.*, (1968) described chemosterilants as chemicals that produce sterility regardless of the method by which the state is attained.

The term *sterility* means inability to produce offspring. The sterile individual cannot contribute a genome to the next generation. The definition however does not include individuals who do produce progeny which, in turn, may be incapable of reproduction. The progeny may inherit a factor from the parents, which render the progeny incapable of reproduction. In 1968 LaBrecque has defined chemosterilants as chemicals that (a) cause sterility by preventing the development of sperms or ova, (b) cause the death of sperms or ova after these have been produced, or (c) so severely injure genetic make up of the sperm or ovum that fertilization, if accomplished will not result in viable progeny.

Methods of producing sterility: Several other methods known to produce sterility are listed below:

(*i*) *Hybridization* — Sterility can be caused by crossing closely related species, races or strains.

(*ii*) *Inferior genetic strains* — Knipling (1940) suggested that inferior insect strains could probably be produced in the laboratory and introduced on a systematic and continuous basis into a natural population in which it might survive long enough to add its inferior genetic characters to that population.

(*iii*) *Genetic means* — If a female belonging to half weak-race is crossed with a male of a strong race, the male progeny will be normal and fertile, while a female progeny will be an intersex and sterile. If males of weak-race are released the females mating with these would produce a generation in which all females would be intersexuals and sterile. The liberation will have to continue for several generations. The phenomenon of chromosome translocation has been known in *Drosophila*, mice and plants. It affects the fertility of the heterozygote and not of homozygote.

Principles of insect control by sterile individuals: are two methods by which a natural population of insects in the field can be made to face adverse effect of sterile individual. In one method individuals of pest species to be controlled are produced in large number artifically in the laboratory, sterilized and released at periodic intervals in the natural fertile population in the field so that sterilized individuals interfere with reproductive potential of pest population in the field. This method involves a lot of expenditure in terms of facilities required for mass production of insects. The second method will involve a procedure by which sufficient number of natural population is rendered infertile by the use of chemicals like chemosterilants to which insects can be attracted by the use of suitable bait.

Theoretical concept of efficacy of artificial release of sterile insects—The significant feature of the insect population control by sustained release of a constant number of sterile insects is that each release achieves a higher and higher ratio of sterile to fertile insects and, therefore, becomes progressively more efficient (Table 1) provided the initial release rate is high enough to cause a decline in natural population. The principle involved is to upset in the beginning itself, the capacity of the insect to increase.

TABLE-1. THEORETICAL TREND OF A HYPOTHETICAL INSECT POPULATION SUBJECTED TO CONTROL BY RELEASE OF STERILE INSECTS.

Assumed natural population of males and females each generation.	No. of sterile males released each generation.	Ratio of sterile to fertile males	Per cent of females mated to sterile male	Male and female progeny produced
1,000,000	2,000,000	2:1	66·7	333,333
333,333	2,000,000	6:1	85.7	47,619
47,619	2,000,000	42:1	97.7	1,107
1,107	2,000,000	1,807:1	99.9	<1

If we assume an insect having capacity to increase five-fold each generation, there is a need for a higher sterile to fertile ratio in order to offset the assumed five-fold increase potential of reproducing insects (Table-2). Allowance must be made for reduced vigor and competitiveness of the organism due to sterility.

The success of sterile-release method depends on several factors *viz.,* (i) method of inducing sterility without adverse effects on mating behaviour and competitiveness of male insects, (ii) practical method of rearing large number of insects, (iii) information on natural population density and the lowest density periods, (iv) less costly methods of rearing and releasing insects and (v) released insects must not cause undue damage to crops or live-stock, or create hazards and nuisance to man and animals.

TABLE-2. TREND OF AN INSECT POPULATION SUBJECTED TO STERILE INSECT RELEASES WHEN NORMAL INCREASE RATE IS FIVE-FOLD

Generation	Natural population increase (5-fold)	Control population		Ratio of sterile to fertile
		Natural population	Sterile population	
1.	1,000,000	1,000,000	9,000,000	9:1
2.	5,000,000	500,000	9,000,000	18:1
3.	25,000,000	131,625	9,000,000	68:1
4.	125,000,000	9,535	9,000,000	942:1
5.	625,000,000	50	9,000,000	180,000:1

Theoretical concept of efficacy of sterilizing the natural population by means of chemosterilants—The method of sterilizing population is basically different from the technique of rearing and releasing of sterile organisms, although both methods involve sterility. The basic requirements for developing and putting each system into practice differ drastically, though these might be integrated to advantage but these are not the same.

Pest population subjected to chemosterilants in the natural population follows the same trend as those subjected to conventional killing agents like insecticides. The big difference between the two is that sterility is potentially more effective because two separate effects are produced *viz.*, (i) sterilized individuals cannot reproduce an effect equivalent to killing in terms of reduced reproduction and (ii) the sterilized individuals can in turn reduce reproductive capacity of the remaining normal individuals thereby giving a bonus effect

This system is a combined chemico-biological method of insect control in which the effect of chemosterilant on the treated individuals would be chemical, and the effect of sterilized insect on mating with normal ones would be biological.

Table-3 shows that sterility technique would achieve elimination of the population by the sixth generation, whereas insecticidal method would do the same in 21 generations.

Advantages of control by sterility methods—(i) The reproductive potential of the population is reduced to the extent sterility is achieved in the natural population. This reduction is the same as achieved by killing.

(ii) The reproductive potential of the pest population is reduced to the extent that sterilized individuals will compete for mates with unsterilized individuals. *Bonus effect* will be equal to initial sterilizing or initial killing effect.

Table-3. Relative Trend of Insect Populations Subjected to (A) No Control (B) 90% Kill With Insecticide in Each Generation and (C) Chemical Production 90% Sterility, Each Generation.

Successive generations		Number of insects resulting from		Sterility
		No control	Insecticide	
1	Parent	1,000,000	1,000,000	1,000,000
2		5,000,000	500,000	50,000
3		25,000,000	250,000	2,500
4		125,000,000	125,000	125
5		125,000,000	62,500	6
6		125,000,000	31,250	0
11		125,000,000	976	–
16		125,000,000	31	–
21		125,000,000	1	–

(iii) Sterilized individuals, through movement, can adversely affect reproductive potenial of the individuals outside the area treated (*space effect*).

(iv) Among long-lived organisms, the sterilized individuals can affect the reproductive potential of the population in subsequent generations (*time effect*).

(v) The sterilized individuals will compete with unsterilized individuals for food and shelter in the environment.

Types of chemosterilants: The first experiments on chemical induction of sterility in insects were done with compounds widely used in cancer research (Borkovec, 1964; Goldsmith *et al.*, 1948; Mitlin *et al.*, 1954). Insects served as convenient test animals in cancer-chemotherapy screening programmes. Until 1964 all compounds effective as insect chemosterilants were largely cancer chemotherapeutic agents as given below :

(i) *Alkylating agents*—These are exceptionally effective as male sterilants. The alkylating agents are sulfonic acid esters, nitrogen mustards and aziridines. Alkylation is a substitution of an alkyl group for a hydrogen atom.

In a heterocyclic compound, any substitution on the carbon ring of aziridinyl chemosterilants decreases insect sterilizing activity. The substitution of one or more alkylating (aziridinyl) group shows that mono-and-poly functional aziridines do not differ as sterilizing agents though the latter are more powerful. Examples are tepa and apholate. Nitrogen mustards are of little importance as sterilants.

(ii) *Antimetabolites*— These female sterilants are structural analogues of purines, pyrimidines and folic acid, interfering with nucleic acid synthesis which is a continuous process in the female adults. The gonads are principal loci of mitotic activity and therefore interaction with the antimetabolite is facilitated. The adult male has already a full complement of mature sperms and so it may be resistant to antimetabolites like aminopterin, 5-fluorouracil *etc.*

(iii) *Radiomimetic chemicals*—Elson (1963) listed alkylating agents, antimetabolites and miscellaneous chemicals like urethan, causing biological effects closely resembling those produced by radiation. A list of 25 biological effects, produced by radiation, fall in four categories namely cytological and genetical, biochemical, pathological and physiological.

Assessment of chemosterilants: The practical application of chemosterilants depends on several factors like the nature of the active compounds, the varieties of ways in which these can be administered and diversity of insect species. The laboratory and field applications are widely different. However, laboratory testing is still the only reliable method of assessing the potentiality of any chemosterilant. Approximately 400 chemicals have indicated biological activity and these have been tested as potential chemosterilants. The methods of testing of chemosterilants are given below.

(i) *Screening*—Between 1959 and 1961, the USDA developed a technique of screening various chemicals on houseflies, Mexican fruit flies and screw worm flies, which were chosen because they could be kept successfully on artificial diets and their life history was short. The diet (consisting of 6 parts suger, 6 parts non-fat dry milk powder and 1 part egg yolk) was mixed with a specific amount of chemosterilant and the effect was evaluated by comparing the number of eggs produced and the eggs hatched with appropriate controls. Detailed techniques for testing chemosterilants on houseflies (Gouck and LaBrecque, 1964), Mexican fruit flies (Shaw and Riviello, 1962) and the screw worm flies (Crystal, 1964) are already known. The purpose of screening is to test as many compounds as possible on different insects and to provide basic information on correlation between chemical structure and activity of compounds. Screening can offer only elementary and incomplete information because the activity of chemosterilants is dependent on the mode of administration, developmental stage of treated insects and species of test insects.

(ii) *Administration*—Chemosterilants can be administered in three ways *viz.*, orally, topically or by injection (Borkovec, 1966). Oral administration is easy and preferable in spite of inherent inaccuracies of the technique. It is the most suitable method of application and is less laborious. Injection is the best procedure for quantitative evaluation of activity but it is limited to water soluble compounds. Topical application is done by dissolving the chemosterilant in a suitable volatile solvent. It offers many advantages such as the most precise quantitative determination of a chemical for sterility although penetration through the biologically active cuticle is variable and it is not practical in inducing sterility in large numbers. However, it is highly instrumental in determining mode of action, breakdown and speed of transfer of the sterilants.

Mode of action of chemosterilant: When both sexes are treated with chemosterilants, partial sterilizing effects are observed in each sex. It is therefore necessary to consider separately the physiological effects on male and females. The chemosterilants are responsible for causing cytogenetic damage which is related to sterility. There are two types of chemosterilants. One group resembles chemical mutagen producing heritable mutation while the other does not produce heritable mutations. Sterility is a condition which can be produced by several ways such as aspermia or sperm inactivation in males and delayed lethal mutations (D.L.M.) in both males and females.

The cytogenetic effect of chemosterilants are less known in comparison to those of radiation. The chemosterilants can react with either one chromatid or with the whole chromosome. There are many other ways in which chemical can affect the genetic material. The initial event is not always chromosome-break. The damage could result in chromosome bridges in the sperm or egg nucleus an unequal distribution to genetic material in subsequent divisions. Under certain conditions, chemicals may even be more powerful in inducing D.M.L. than X-rays. The majority of alkylating chemosterilants are known to be chromosome breakers.

The physiological effect of gonads are considered at three levels *viz.*, (i) gross effects on the reproductive organs and gametes, (ii) cytogenetic effects and (iii) the molecular effects. The great majority of published papers are concerned with the first two aspects.

Effect on femals: The basic unit producing eggs is a pair of ovaries. These are partially developed in newly emerged adults. The interference of chemosterilants with their oogenesis and maturation can be followed easily. High mitotic activity and the growth of the ovaries facilitate cytological and microscopic observations.

Gross effect on ovaries: The effect of application of chemosterilants is that no eggs are laid, and if laid, the zygote is developed into a mature offspring. The chemosterilants interfere with ovarian growth. Certain chemosterilants like thiotepa are so powerful as to sterilize fully developed eggs of *Aedes aegypti* which were already fertilized by an untreated male (Bertram *et al.*, 1964). The age of the female, physiological condition of the ovarioles and nature of the chemosterilants are important factors. The histopathological effect of chemosterilant of female insects is retardation or complete cessation of ovarian development in houseflies. (Hair and Adkins, 1964; Miltin and Baroody, 1958; Morgan and LaBrecque, 1962, 1964; Nayar, 1963), mosquitoes (Murray and Bicklay, 1964; Rai, 1964) fruit flies (Keiser *et. al.*, 1965), *Drosophila* (Cantwell and Henneberry, 1963) and almond moth (Gangrade and Pant, 1970 c). The changes ranged from complete necrosis and disappearance of the organ to a slight reduction in its size. The treated females crossed with untreated males produced fewer eggs (Gangrade and Pant, 1970 a) or none (Simkover, 1964) and there was negligible hatching. Inhibition of ovarian development was the most characteristic feature of female chemosterilants and the fecundity was greatly reduced because of the retardation of ovaries.

Cytogenetic effects—Cytological studies on the housefly (Morgan and LaBrecque, 1962) and almond moth (Gangrade and Pant, 1970 a) showed that chromatin in the nurse cell nuclei was clumped. The chromatin of the oocyte nucleus was also clumped in the almond moth in which the region of ovarioles containing differentiated and undifferentiated oocytes and nurse cells were severely damaged. The general organization of the ovarioles of almond moth was disrupted due to apholate feeding, although the fully matured oocytes were apparently unaffected (Gangrade and Pant, 1970 c).

Molecular effects—Detailed metabolic pathway of chemosterilants is unknown in insects or any other animals. Most of the alkylating agents, antimetabolites the chemosterilants distribute themselves evenly through the whole organism to which they are administered (Hays, 1964; Nadkarney *et al.*, 1959). No selective accumulation in the gonads of insects was observed (Chang *et al.*, 1966) but it did in the testes and not the ovaries of the boll weevil treated with C^{14} labelled tepa (Hedin *et al.*, 1965). Specific effects of apholate were noted in the housefly such as inhibition of DNA synthesis in the eggs and inhibition of lactic dehydrogenase enzyme (Kilgore and Painter, 1964). They noted incorporation of 5-fluorouracil into the RNA of housefly eggs.

Effect on male insects: The male adults may become less competitive and less vigorous as compared to normal males. In the almond moth, the number of matings accomplished by chemosterilized males was 1 to 6 as against 1 to 7 by normal males and the sterilized males were quite competitive (Gangrade and Pant, 1970 a).

Gross effects on testes—Unlike ovaries, testes do not suffer any morphological changes. The testes show various degrees of atrophy or inhibition due to treatment with alkylating agents in Mexican fruit flies (Shaw and Rivielo, 1962), *Drosophila* (Canwell and Henneberry, 1963), mosquitoes (Rai, 1964), boll weevils (Lindquist *et al.*, 1964) and almond moth. Male sterility was seldom due to aspermia. The sperm of 48-hour treated males of almond moth did produce some progeny on mating with untreated females and none in other cases although the sperms were normal in appearance. Whether the sperms of treated males of almond moth contained any D.L.M. could not be ascertained (Gangrade and Pant, 1970 a).

Cytological effects—The susceptibility of the spermatogonia, spermatocytes, spermatids and spermatozoa varies. When the chemical affects spermatozoa, the sterility induced is temporary. If immature stages are affected, the fertility is regained after few matings. The effects of radiation and chemosterilants are similar and dose dependent. All stages are equally sensitive. Chromosome tranlocations and aberrations were produced in *Drosophila* (LaChance and Riemann, 1964; Rai, 1964).

Molecular effects—Interaction with sperm nucleic acids (DNA) is probably one of the effects. Another possibility is the transfer of untreated alkylating agent with the semen and its reaction with the unaffected sperms. The possibility of such a physical transfer of the chemosterilant or its metabolite was shown in boll weevils (Lindquist and House, 1957) and in almond moth (Gangrade and Pant, 1970 b). Hedin *et al.*, (1967) detected radioactivity in the spermathecae of the females mated with treated males thus giving presumptive evidence that the sperm was labelled. This is called *stored sperm effect*. Still another is a depurination theory of DNA, *i.e.*, detachment of alkyl group which is a slow process and may occur after several days or weeks of storage of sperms.

Field development and evaluation of chemosterilants: The field testing of possible methods of utilizing chemosterilants against natural population in order to test their feasibility for control or eradication, is very important. This research involves field studies. In release-method chemosterilants can be used in place of radiation because former is more effective, economical and easier to use. Current possible methods of using chemosterilants are (a) release of sterile insects into natural populations, (b) combination of chemosterilants with attracants, baits or lures for treatment of natural populations, (c) direct treatment of natural population and (d) integration of the use of chemosterilants with other control methods.

Most chemosterilants are hazardous and highly toxic. Field experiments with these materials should not be conducted until some compounds are known which will be non-hazardous to any form of life. So far no chemosterilants have been discovered which can be recommended for large scale field applications.

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29

BIOLUMINESCENCE IN INSECTS

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THE ability of certain animals to produce light is known as bioluminescence, a phenomenon which exists in more than half of zoological phyla. It is highly developed in class Insecta and some deep sea fishes. The light production is the result of a chemical reaction that does not involve production of heat and is brought about with the half of a suitable substrate and an enzyme-system. In some animals notably certain marine fishes, luminescence is due to the presence of luminescent symbiotic bacteria. In insects the production of light is always a phenomenon of chemiluminescence. The examples of true or self luminescence are found in Collembola, Homoptera, Diptera and Coleoptera. Coleoptera constitutes the largest group in which several hundred species are known to contain highly developed photogenic organs. These insects are known by various common names like fireflies, glow worms, lighting bugs, blinkies *etc*. The best understood luminous group of insects are fireflies belonging to the family Lampyridae and Elateridae.

The phenomenon of bioluminescence is also found in plant kingdom but is confined only to bacteria and fungi. Saprophytic luminous bacteria are widely distributed in the sea where they grow on dead fish. They also can be found on stored meat and on the flesh of dead animals. Luminous fungi are also very common. In certain forms of mushroom, both fruiting body and mycelium may be luminous. When mycelium of luminous fungi penetrates dead wood in forest it gives rise to phosphorescent wood or *foxfire*. The entire wood glows in the dark due to invading mycelium.

Light emission in animals is almost always the result of nerve stimulation. Animal kingdom has several groups which emit light *e.g.*, snails, squids, crustaceans, insects, fishes *etc*. In some animals emitted light is diffused while in others there are present accessory structures for focusing the light by reflecting surfaces and lenses.

Organs of light production in insects: The location of light organs in insects is very variable but these are always situated very close to body surface behind a window of translucent cuticle and are usually different in male, female and larva. In size, the organs vary from minute pin heads to masses (Fig. 29-1). These are found from head to the tip of abdomen

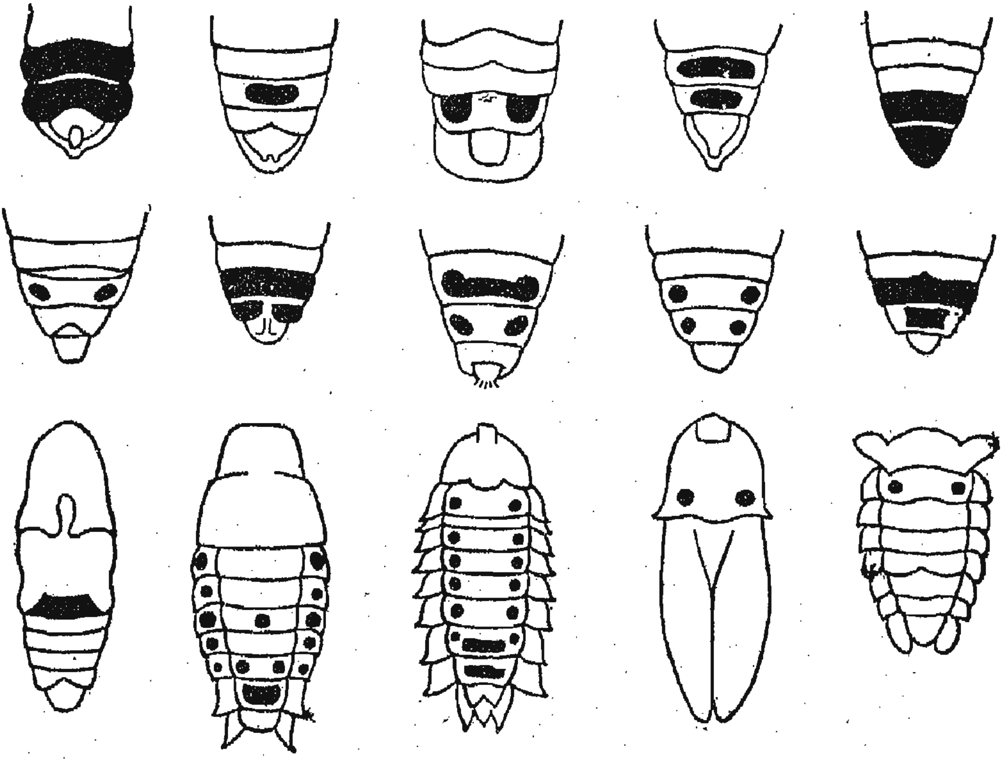

Fig. 29-1. Diagrams showing position and size of photogenic organs (dark areas) in representative fire-flies.

including thorax. In *Pyrophorus* (Elateridae) two kinds of luminous organs are present. There is one pair on the posteriolateral margin of prothorax and the other on the posterior abdominal segments. In *Photinus* (Lampyridae) these are present on the ventral region of sixth and seventh abdominal segments in adults and eighth segment in larvae. These organs consist of large number of cells called *photocytes* located just beneath the integument. The cuticle in these regions is transparent to permit the light to pass. Behind the photocytes there is a reflecting surface chiefly consisting of urate granules. The photocytes are richly supplied by tracheoles and contain a heavy aggregation of mitochondria.

Types of light emissions: There are four types of light emissions giving different glow-patterns as described below:

Continuous glow — Light is given out without interruption in a continuous glow of uniform intensity throughout life as in lower organisms like bacteria or in eggs and pupae of some fireflies or in larviform females of *Phengodes*. Often the glow fluctuates under some mechanical stimulation.

Intermittent glow — Here the glow lasts only for a few seconds. In *Photuris* larva the light appears and disappears in a second. Intermittent glow is under nerve control and the intensity of light increases under certain stimulations given by increased activity or some other external conditions.

Pulsation — *Pyrocoelia and Luciola* emit light in pulses ranging respectively, 6 to 13 and 60 to 110 per minute. Often pulses are fused, giving effect of a continuous light.

Flash —This is the most common type of light emission found in lampyrids (*Photinus, Photuris, Luciola etc.*). In its simplest form flash consists of a burst of light which remains only for a short duration. Unlike, as in continuous glow, the emission is of much greater intensity. The light intensity in a flash rises abruptly from zero to a maximum and declines suddenly back to zero. Duration of flash is of the order 0.1 to 0.2 seconds. In this type of emission there is only one peak of maximum intensity; however in certain insects light is flashed with multiple peaks. In many fireflies the frequencies of flashes act as mating signals to the opposite sex. These signals are remarkably precise in their time relation and differ characteristically in different species. It has been found that the males of about dozen species of fireflies which may be active at night in the field at the same time, can be identified reliably from observations of their flashing pattern and frequencies.

Physiology of light production: *The light is produced* as a result of interaction between a substance called *luciferin* and the enzyme, *luciferase*. Different organisms have different compounds for light production and therefore one can expect that the nature of luciferin will be different in different animals. Several luciferins have been isolated in more or less pure forms from a wide variety of organisms. The luciferin of bacteria is an aldehyde complex of reduced riboflavin-5-phosphate and its luciferase is therefore a flavin-enzyme. The luciferin of firefly is chemically different. It is 2-(4-carboxythyzol)-6-hydro—xybenzothyzol which is acted upon by ATP (adenosine triphosphate) under the catalytic influence of luciferase. Still other luminescent system exists in *Cypridina* (a small marine crustacean) in which the luciferin is a polypeptide linked to a yellow chromophore. This substance is discharged to the exterior along with luciferase where photogenic reaction takes place in the presence of oxygen. The most simple system is found in jelly fish where luciferin is a protein which emits light in the presence of calcium ions.

Biochemistry of light production has been studied in very great details in insects notably fireflies in which luciferin is secreted by specialized cells called *photocytes*. When a suitable nerve impluse reaches the photogenic organ the nerve endings produce acetylcholine which is quickly hydrolyzed to form acetic acid and choline. ATP and co-enzyme A present in photocytes now react with acetic acid forming pyrophosphate, adenylic acid and acetyl co-enzyme A. The luciferin now combines with adenylic acid to form a substance called adenylluciferin which in the presence of luciferase is oxidized to form excited or high-energy-adenyloxyluciferin which immediately decays to a low-energy-adenyloxyluciferin with the production of light. This reaction is so efficient that the substrate when metabolized produces 98 per cent of energy in the form of light and there is practically no rise in temperature.

The nervous system of insects is intimately connected with the functioning of light organs and if the insect is decapitated or a nerve supply to the organ is cut, the flashes immediately cease. If such an insect is electrically stimulated or the photogenic organs are given direct stimulus, the light is again emitted so long as the nerves are excited. Insects under the influence of a nerve poison stop emitting light.

Biological significance of light production: In primitive forms of life as in bacteria, cases are known where light may be produced as a result of one of the side reactions during certain chemical activity within the cell. In such cases, it probably has no biological significance. In higher forms of life however, it has a purpose for the animal species which produces it. The light is known to act as a mating signal in glow worms and fire-flies. In certain other species the bioluminescence attracts individuals of the same species to aggregate at one spot which besides other things, facilitates and increases the chances of mating. In certain glow worms the females are apterous., the light production, therefore, is a very vital phenomenon to attract the winged members of the opposite sex.

The most unique example of light acting as a lure to the prey is found among the luminous larvae of a dipteran, *Bolitophila* commonly known as New Zealand glow worm. The larvae of this fly emit bluish green light from the thoracic organs formed by the free ends of Malpighian tubules lying just beneath the transparent cuticle in the thoracic region. *Bolitophila* is the only known example among insects where Malpighian tubules have taken up photogenic function. The female fly deposits eggs on the ceiling of dark caves. The maggots on hatching hang down by a sticky silken thread, two to three feet long. During night the entire upper portion of the cave glows with the light attracting the flying insects from outside. These insects get entangled in the sticky threads and are preyed upon by the larvae. As the larvae of *Bolitophila* grow the intensity of glow decreases and finally disappears in the adults. These caves inhabited by flies are popularly known as luminous caves and are great tourist attraction in New Zealand.

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THE MECHANISM OF DIFFERENTIATION

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The process of the development of different cell types from a common parent cell is termed differentiation. In all multicellular organisms, out of a simple-looking egg emerges a complex organism, complete and perfect in every detail. The set of genes present in the egg is naturally duplicated each time the cell divides and therefore every cell whether in nerve, muscle, epithelium, or bone possesses the same set of genes. How is it that the undifferentiated cells derived from a cleaving egg grow into the cells of heart, liver, nerve, muscle or bone, each kind specialized in its own way, both morphologically and physiologically.

The riddle of differentiation has not been solved but there is great progress mainly due to rapid advancement in biochemistry, the development of new techniques and proper choice of experimental organisms. Formerly, the three variables *viz.*, cell nucleus, cell cytoplasm and cell environment were studied in isolation but the scientists today are concerned with the inter-relationship of these variables as the development proceeds. As a result of such type of studies, a biochemical basis of differentiation has been postulated, according to which enzyme induction or enzyme repression of certain genes plays major role. Unmasking (turning on) or masking (turning off) of certain genes in particular groups of cells at very specific times during the course of development leads to the difference in their final form and function.

The following two examples reveal the influence of cell environment in differentiation.

(i) Differentiated cells removed from their usual environment tend to de-differentiate when grown separately in tissue culture experiments.

(ii) Hydra which in normal environment reproduces asexually by budding, develops gonads and sex cells if carbon dioxide concentration of its aqueous environment is significantly elevated. Apparently the carbon dioxide concentration determines when a normal hydra cell will differentiate into sex cell.

It is possible that even small differences in the chemical environment (available oxygen, carbon dioxide, nutrients and substances secreted or excreted by neighbouring cells) of the cells of a developing embyro may be responsible for differentiation by influencing gene action.

Important relationship between certain regions of egg cytoplasm and subsequent cell differentiation has been well established in several invertebrates and vertebrates. Visible and/or invisible gradients or localized regions have been clearly demonstrated in the egg cytoplasm of many organisms. Studies of cell lineage and of consequences of experimental alterations in the egg organization by centrifugation or by extirpation have unequivocally proved the morphogenetic importance of the gradients or the localized regions in the egg cytoplasm. It is known that the gradient or localization is established in part during oogenesis and in part by the rearrangement of egg material during and after fertilization (Conklin, 1905). Nucleocytoplasmic interactions have been reviewed by Weismann (1893), Wilson (1925), Morgan (1934), Briggs and King (1959), Brachet (1960) and Gurdon and Woodland (1968).

The following examples selected from studies on insects show the influence of cytoplasm on nuclear activity and help in understanding the mechanism of differentiation.

Cytoplasmic localization actually affecting nuclear activity has been amply demonstrated in gall midges (Cecidomyidae, Diptera). Adult gall midges are unusual in that they have larger number of chromosomes in the germ cells and less number in somatic cells. Studies on *Miaster* and *Mycophila* (Nicklas, 1959, 1960), *Wachtliella* and *Rhabdophaga* (Geyer-Duszynska, 1959) and *Mayetiola* (Bantock, 1961, 1964) have shown that an intimate relationship exists between the development of germ cells and the polar egg cytoplasm in these insects. In *Mayetiola destructor* zygote nucleus has 40 chromosomes; the nucleus divides as usual and when eight nuclei are produced, one moves to the posterior pole where it gets surrounded by the polar cytoplasm or germ plasm. At the next division 16 cleavage nuclei are produced, 2 located in the germ plasm and 14 distributed throughout the egg cytoplasm. A constriction occurs in the region of posterior pole and separates two primary germ cells from the remaining egg cytoplasm that has the other 14 somatic nuclei. The fifth cleavage division is peculiar; only the 14 somatic neuclei divide and that too in such a way that each of the 28 daughter nuclei has only 8 chromosomes instead of the usual 40. During this division, 32 chromosomes are eliminated from all the somatic nuclei but the germ nuclei neither undergo division nor lose chromosomes; each germ cell nucleus retains 40 chromosomes. It is evident that the polar cytoplasm (germ plasm) protects the germ nuclei from the influence of the surrounding cytoplasm, an impression that is strengthened by the formation of cell membranes cutting this pair of cells off from the rest of the egg. Thus it is a clear example in which the presence or absence of a cytoplasmic factor determines the behaviour of the nuclei during the fifth cleavage. Before the entry of cleavage nuclei of primary germ cells if the region of polar cytoplasm is subjected to ultraviolet radiation or centrifugal force certain changes occur and later when the cleavage nuclei arrive, they like somatic nuclei lose 32 out of 40 chromosomes, apparently because the polar plasm has lost the protective function due to the changes brought about by the treatments. A similar effect is seen if the germ nucleus is artificially prevented entry into the polar region by ligaturing it off from the rest of the cytoplasm.

Two components, a non-granular and another granular, have been recognized in the pole plasm. The granular component is believed to be an aggregation of ribosome-like particles embedded in finely fibrous material (Mahowald, 1962; Ullmann, 1965). There is evidence to support that these represent two separate factors. The non-granular component of the pole plasm can induce even somatic nuclei to become germ cells to a limited extent; these cells resemble primordial germ cells in every way but are unable to complete gametogenesis. The granular component on the other hand, prevents chromosome elimination and thereby permits the final stage of gametogenesis.

Just as the germ plasm prevents chromosome elimination in the gall midges, it probably plays a comparable role in other organisms by protecting the germ cells from specialization and loss of totipotency. A germ plasm of similar chemical composition has been reported in frogs in which too the irradiation of germ plasm causes partial or total sterility.

The influence of cytoplasm is not confined to early embryogenesis. Studies on the grasshopper, *Chortophaga viridifasciata* has shown that the differences in nuclear morphology and cell functions are under cytoplasmic control (Kawamura, 1960).

Observations and experiments on *polytenic* (giant) chromosomes (found so far only in certain dipterous insects) indicate the possible mechanism of cell specialization during early differentiation. Each one of these chromosomes is comparatively thick and long and shows large number of dark chromatic and light non-chromatic bands. Definite portions (dark bands) of one or more of these chromosomes swell up to form *puffs* or *Balbiani rings*. It has been found that the puffs are sites of strong synthesis of RNA and proteins (Beermann, 1952; Pavan, 1958).

It has been reported that the occurrence of puffs follows a specific pattern. Different genes (dark bands) puff up and become active at different stages of development of an organism. In young larvae certain gene sites puff up while in mature larvae other gene sites of the same or other chromosomes puff up.

Further, the activity of the gene site is not only specific with respect to age but also with respect to cell type. Two types of secretory cells occur in salivary glands of chironomids. Each type of salivary gland has its own pattern of puffing which also undergoes changes during development.

Results of nuclear-transfer experiments, whereby nuclei from salivary glands of advanced larvae were transferred into preparations containing the cytoplasm of developing eggs, further confirm that specific factors in the cytoplasm are responsible for activity of only certain genes. In these experiments, puffs from original sites disappear and the new environment induces puff formation at fresh sites on the same or other chromosomes. The disappearance of puffs at certain sites and then formation at other sites is analogous to the turning on one set of switches and turning-off the others. Thus, the studies on polytenic chromosomes provide a working model of the possible mechanism of cell differentiation even during early development.

The precise nature of the cytoplasmic factors and the mode by which they transmit specific information to the gene sites have yet to be revealed. However, a number of successful nuclear transplantation experiments in amphibian eggs have opened up a new chapter. In one set of experiments by Gurdon and Woodland (1968), nerve cell nuclei from adult brain or embryonic cell-nuclei from midblastula stage were transferred (i) to enucleated unfertilized egg, (ii) to enucleated growing oocyte and (iii) to enucleated maturing oocyte. A synthetic egg or oocyte was thus produced in which the cytoplasm belonged to a cell whose nucleus was in a stage of activity opposite to that of the donor nuclei. The synthetic egg or oocyte was then incubated in a medium containing labelled precursors of either DNA or RNA. It was found that original activities of the donor nuclei cease and they begin to function like the displaced host's nuclei and also the donor nuclei swell up increasing 2 to 50 times in diameter. The results provided a direct support in favour of the earlier postulate that nuclear functions and gene activities were dependent upon the dictates (signals) of normal constituents of living cytoplasm.

Furthermore, swelling or enormous increase in diameter of the donor nuclei indicates a possible pathway by which the signals are transmitted from cytoplasm to the gene sites. The following facts are relevant in this connection: (a) the chromatin (which contains the genetic material in the nucleus) is believed to become dispersed during swelling, (b) some of the cytoplasmic proteins enter into the enlarging nuclei (Arms, 1967) and (c) a close temporal relation exists between the passage of cytoplasmic protein into enlarging nuclei and the initiation of DNA synthesis. These facts have led to the interpretation that the nuclear swelling facilitates entry of the regulatory cytoplasmic molecules which then easily come in association with the dispersed chromatin. Changes in gene activity are then conveniently induced by regulatory molecules once these reach and get in touch with the chromatin material. Of course, the kind of change in the gene activity will be determined by the nature of the cytoplasmic molecules that enter nucleus. The regulatory cytoplasmic components themselves have been hardly identified but there is an indication that these are not species specific. Liver nuclei from adult mouse, when transplanted into enucleated frog's egg, are induced to synthesize DNA by the latter's cytoplasm (Graham *et al.*, 1966). This in short, is the current concept of the possible mechanism by which gene sites are activated or repressed by regulatory cytoplasmic molecules resulting in *differentiation*. It has now also been clearly demonstrated that the egg cytoplasm exerts its morphogenetic effect via the nucleus by bringing about changes in chromosomal behaviour and consequently also in nucleic acid synthesis.

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31

MICROTOMY—A HISTOLOGICAL TECHNIQUE

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MICROTOMY is the technique of cutting animal or plant tissues into minute slices and preparing them for histological studies. It consists of several different procedures like fixation, dehydration, clearing, embedding, sectioning, staining and mounting series of sections of the parts to be examined.

Fixation of the material: Before the tissue can be histologically examined these must be fixed suitably. *Fixation does not mean fixing the tissue on a substrate.* In histological terminology, it implies two steps : first, the *rapid killing* of the living tissue so that it may not have time to change the form it had during life, in other words no or very little post-mortem changes should occur; and second,, the *hardening* of tissue to such a degree as may enable it to resist further change of form due to the action of the reagents with which it may subsequently be treated. Without proper fixation it is impossible to get good preparations.

Tissues can be killed suddenly by application of *heat* which is often done by immersing the whole organism (if these are small *e.g.,* insects) or their organs into boiling water or other suitable chemical fixative at 80 to 90°C. By the latter method a sudden killing is brought about and at the same time tissues are fixed without undergoing appreciable post-mortem changes. The time of exposure and the temperature will vary for different objects and these are to be standardized by preliminary trials.

Large animals are often first *narcotized* by chemicals like chloroform, benzene, ether, alcohol, cocaine *etc.* One of the disadvantages with this method is that chemicals like ether and chloroform are extremely injurious to cells which undergo distortion. Large animals like cat and dog are first anaesthetized in coal gas chamber and killed by hard blow on the head and then histological preparations of required organs are made by removing them from the body. Fortunately small size of insects makes it easy to apply methods of sudden killing very efficiently. Some histologists prefer to dissect the insect alive in physiological saline and remove the organs for subsequent preparations.

After the tissue or the organism is "killed", these are treated with certain fixative agents. The objective of this treatment is (i) to impart to the tissues the degree of hardening which enables them to resist the changes which otherwise would have been brought about as a result of further processing, (ii) to render insoluble the elements of the cell that would otherwise be dissolved off by the liquids employed in subsequent treatments and (iii) to produce optical differentiation in the structure. The fixing agents alter the refractive indices of the different substances thus making the cell more suitable for optical examination under a microscope. The choice of fixative is guided by the nature of the tissue and the type of the study to be made. For gross histological studies a single fixative is often used but in special cases, different fixatives are employed for fixing different cell constituents. In such types of treatments the fixatives employed have high degree of specificity to different parts of the cell.

Duration of the application of fixative may be unimportant or highly critical depending upon the type of tissue and the nature of fixative. The fixative containing chloroform, for example, renders the tissue unduly hard if it remains in contact for a little longer period than necessary. Besides this, temperature also plays a significant role in the entire process of fixation. Normally, room temperatures are quite suitable for the action of fixative agents on the tissue but sometimes temperature of the fluid is raised to increase their efficiency particularly the penetrating power.

Mode of the action of fixatives—One of the important actions of fixation is to bring about stabilization of cell protein and when these agents are added the protein sols undergo a change due to an aggregation of protein molecules into microscopic granules. In other cases instead of this type of coagulation, protein sol is rendered more viscous to form gel and the protein gel becomes more solidified. Different cell proteins are reacted upon differently by the fixative *e.g.*, reaction will be different with nucleoprotein than that with albumen. Picric acid, mercuric chloride and chromium trioxide bring about differential coagulation in two proteins, albumen and nucleoprotein.

Almost all fixatives invariably bring about shrinkage of the tissues. It has a harmful effect because it involves distortion in the structural details and adversely affects the optical differentiation of cell material. Shrinkage may also occur when tissues fixed in aqueous fixative are transferred to alcohols and later to xylol for dehydrating and clearing. In case of animal tissues the shrinkage is uniform and this does not bring about much distortion in the overall appearance of the specimen. In plant material, careless dehydration and clearing will lead to the shrinkage of the cell material but not the cell wall, with the result the cells show as if the cytoplasm has been pulled away from the cell wall. In certain cases, however, instead of shrinkage, cells may actually swell up depending upon the osmotic nature of the fluid. The effort of the histologist is always to keep the shrinkage minimum by the judicious choice of ingredients in a fixative.

Besides *coagulant fixatives* described above certain chemicals act as *non-coagulant fixatives* which include formaldehyde, osmium trioxide, potassium dichromate and acetic acid. Formaldehyde does not coagulate albumen but it reacts with it in such a manner that this protein is rendered non-coagulable by alcohol and insoluble in water. The formaldehyde reacts with the NH_2 group of certain amino acids forming methyl bridges which link the protein chain together. Formaldehyde also acts as a good preservative for cell lipids including phospholipids if the duration of treatment is not unduly prolonged.

Due to the differential actions of the various individual fixing substances, often referred to as primary or unmixed fixatives, it is better to use *fixative* mixtures (containing coagulating and non-coagulating type of substances) such as Bouin's fluid which contains formalin, picric acid and glacial acetic acid. Before processing the material for dehydration, it should be thoroughly washed in water or alcohol depending upon the nature of fixative so that traces of excess fixative are removed. This removal is further carried out when the tissue is undergoing the process of dehydration in different grades of alcohol.

Dehydration: This process means the *removal of water* from the tissues. It is necessary to remove the traces of free water contained in the cell or in the fixative mixtures because most of the media in which the specimens are ultimately mounted, are not miscible with water. Further, presence of water would bring about decomposition and thus material cannot be preserved for long time. Faulty dehydration is the commonest cause for poor results, such as shrinkage and deterioration of histological details. The classical method of dehydration of tissues is to pass the object through a graded series of alcohols *i.e.*, 30, 50, 70, 90 per cent and finally the absolute alcohol.

The dehydration with alcohol can be carried out in corked or glass stoppered phials or in cavity blocks with glass tops. In humid weather corked bottles should invariably be used to prevent alcohol from absorbing water from the atmosphere. The period of immersion of the tissue in the various grades of alcohol obviously vary with the size and nature of the material being used. Alcohol has got hardening effect on the material, therefore it is necessary to shorten the duration of treatment in 90 per cent and absolute alcohol.

An alternative substance for dehydration is dioxane (diethylene oxide). It is a very good solvent and mixes well with water, alcohol and paraffin. The material therefore can be transferred from fixative to dioxane and then directly to wax without passing through a clearing agent because dioxane does both dehydration and clearing simultaneously. It is a good method of dehydration since it is simple, produces excellent results if a proper grade of dioxane is used and permits a flexible schedule of application. The object can be left for months in dioxane. It has little or no hardening effect upon the material as strong alcohol and xylol do. It produces good preparations even in the hands of the beginner. In spite of having these advantages, its use is limited due to its toxic nature. It is a dangerous substance and has a marked physiological and cumulative action on human beings.

Dealcoholisation of clearing: Dealcoholisation of material is essential because the resinous media used for mounting and the waxes used for embedding are not miscible with alcohol. Clearing agents generally have a high index of refraction which makes the specimens translucent and facilitates the penetration of waxes and mounting media. Dehydrated material should not be taken out of alcohol, instead the clearing agent should be added to it. If the object is taken out of alcohol for transferring it for clearing it may float on the surface of dealcoholising fluid and can undergo shrinkage. The object is considered perfectly cleared when the wavy refraction lines caused by the mixture of the two liquids at their interfaces have ceased to form. The material should not be embedded until this has first been soaked for some time in a fresh quantity of clearing agent to remove all traces of alcohol.

For paraffin embedding, cedar wood oil and methyl benzoate are the best dealcoholising agents. Of course people have been using clove oil and xylol also but there are certain difficulties with these materials. Clove oil mixes with paraffin and renders the tissues brittle quickly and xylol used alone causes shrinkage in the material and it is very difficult to dealcoholise completely the material with xylol alone in moist weather. Methyl benzoate removes the last traces of water left after dehydrating in ethyl alcohol and also clears the material beautifully. Cedar wood oil is also very close in all respects to methyl benzoate but it has got advantage that the material can be kept in it for any length of time without any harm.

Paraffin embedding: The embedding or infiltration is the method by which the delicate tissues are surrounded and impregnated by a supporting material like paraffin wax. The other embedding media are ice, gelatin, various waxes, nitrocellulose and special types of plastics. Embedding serves two functions. Firstly, it surrounds an object too small or too delicate to be held firmly by the fingers or by any instrument without any injurious pressure so that by cutting sections through the composite body, the included object may be cut into sufficiently thin slices without distortion. Secondly, embedding mass fills the natural cavities of the object so that the membranes or other structures contained in them may be duly cut *in situ* and even the minutest details of the object are maintained. The process of embedding prepares the material for cutting it into thin sections in a microtome.

After clearing, the next step is to substitute paraffin for the clearing medium. For this purpose molten paraffin wax is used. The material is either transferred directly to the molten wax or it may be passed through a graded series of wax-solvent mixtures. The first method can be used if the clearing agent is cedar wood oil and second if it is xylol. The material should be kept in pure molten wax for complete penetration of paraffin in the tissues. There is no method of determining how long the object will take for proper impregnation with wax. The time may vary from one to several hours depending upon the nature of the tissue.

For embedding purposes several commercial waxes of different melting points are available. The choice of type of wax to be used will depend upon the material and the prevailing temperature conditions. Hard objects require harder paraffin while soft ones need softer wax. When the room temperature is low as in winter, the wax of low melting point should be used.

After impregnation the material is embedded in a block of solid wax. The block can be made in a deep watch glass, porcelain dish or in rectangular paper boat. The containers are first smeared with glycerine and then the molten paraffin is slowly poured into them against the clean glass rod. This is an important step for avoiding air bubbles going in the molten wax. If still there are a few bubbles, a section lifter is gently warmed over a spirit lamp and passed along the molten wax in the container. In this way any entrapped air bubble will escape out. Now the object is lifted with a warm forceps or section lifter and is placed in the container and orientated according to a chosen plan. It is important to ensure that the material must be covered with wax from all sides and there are no air bubbles around it. Often, while placing the object into the molten wax air bubbles stick to its surface. These can be removed by touching them carefully with warm needle. Care should also be taken that the object lies in the middle of the block. Some workers therefore prefer to place the paper boat or container over a thin film of cold water so that the bottom portion of molten wax in the container solidifies quickly and the material is prevented from sinking to the bottom of the container.

Next important operation is to cool the paraffin wax rapidly. If this is not done, wax crystalizes making the block opaque at several places. It is a good policy to let the upper layer solidify and then slowly put the container in cold water. If glycerine has been properly applied to the container, the solidified block will come out easily. The materials once embedded can be kept for any length of time until these are used for cutting sections. Paper boats have one advantage that it is quite easy to write description of the material and direction of orientation on them making it easy to identify the material even if it is cut after a long interval.

Before mounting on microtome holder, the block is trimmed suitably into a rectangular piece of suitable size. Before trimming, the plane in which the sections are to be cut should be determined. Trimming should be done by removing wax in very thin slices with the help of a sharp safety razor or scalpel, leaving the largest amount of wax on the surface which is to be attached to the holder. It is essential that the shape of the block must be rectangular, the two opposite sides must be parallel otherwise the ribbon will not come straight during section cutting. The final trimming of the block can be done by rubbing it over a blotting paper.

Attaching block to the block-holder may appear simple process but in practice it has been found that if this step is not properly attended to, the entire process of section cutting may go wrong. The surface of carrier should first be covered with molten or semi-molten wax and if the wax does not firmly attach, it should be pressed firmly with a heated spatula and if necessary, extra amount of wax should be added on the surface. The trimmed block should then be placed at the centre of the carrier in a cavity made in the mass of wax covering the holder. With the help of heated spatula the block is properly attached so that the wax surface of the carrier is continuous with lower portion of the block. The block holder along with the object is then cooled in cold water. The material is now ready for sectioning.

Sectioning: The mounted block on its carrier is inserted in the jaws of the object clamp and screwed tightly in place. A microtome knife or razor-blade-holder is put in knife holder and fastened firmly and adjusted in a such way that it is tilted slightly towards the microtome. The angle will vary somewhat according to the type of tissue and can be changed as required. The block and the knife should be adjusted in such a way that the lower edge of the block is parallel to the knife edge and its face is perpendicular to the block and it is always at a distance less than a millimetre from the face of the block. The indicator is set to cut the sections at 6 μ which is a good thickness for general work. The locking device of the microtome is released to start section cutting operation. This is continued until sections begin to come off. The first sections should be of paraffin only. By the time sections are complete and regular, these should form a straight ribbon. When the ribbon becomes sufficiently long, its free end is supported by a brush and held by the left hand and if necessary the whole block can be cut in a single unbroken ribbon or ribbon can be cut into short lengths and spread on the paper in serial order.

Before taking up the process of spreading the sections, the slides must be cleaned by cleansing fluid. If the slides are not cleaned, the sections will not adhere to the slide during subsequent treatments.

An affixative (usually Mayer's albumen) is smeared on *the slide* as a thin film. After smearing Mayer's albumen water is placed on the slide, and then the segments of ribbon are floated on the surface of water and the slide is put on the stretching plate until the sections have extended fully. The slide is then removed from the plate and sections are arranged neatly on the slide and dried for further processing.

Staining, dehydrating and mounting: The process involves two steps: the first is the removal of paraffin by xylol and transference to water, known a *deceration-hydration schedule;* the second is the preparation of the stained slide for mounting known as the *dehydration-clearing schedule.* For these schedules reagents should be prepared in two series and stored in numbered and labelled bottles. Each label of deceration-hydration schedule should be marked *down* and each label of dehydration-clearing schedule should be marked *up.*

Alcohol series may be made up with either 90% alcohol or rectified spirit. The following is a typical schedule for Delafield's haematoxylin and eosin staining.

Down series→Xylol→xylol→absolute alcohol→90%→70% 50% → 30% → distilled water → stain with haematoxylin → acid water → tap water.

Up series → 30% alcohol → 50% → 70% → 90% → stain with eosin in 90% → 90% → absolute alcohol → xylol → xylol → mount in Canada balsam.

Staining methods may involve single, double, triple or more strain requiring a whole series of dyes. In these methods the stains may be applied one after another or in some cases the staining solution will contain two or more dyes. Here only double staining by Delafield's haematoxylin and eosin, are described.

The paraffin sections are decerated and hydrated in *down series* and brought to water. In each jar of this series, five minutes are sufficient except in xylol where it should be assured that the wax is dissolved off completely. To hasten this, slides can be slightly warmed on a stretching plate.

Sections are stained in haematoxylin for 3 minutes. The intensity of stain should be checked under microscope and if necessary, the destaining can be done by immersing the slides in jar containing acid water (distilled water with one or two drops of concentrated HCl). The acid water helps in differentiation. As soon as the desired intensity of the stain is achieved, the slides should be immersed in tap water which is alkaline. The treatment with tap water will make the haematoxylin stain dark blue. A good method to determine the differentiation is that only the nuclei should contain haematoxylin stain and the cytoplasm should be colourless. The cytoplasm later on is counter stained with 5 per cent eosin in 90 per cent alcohol while going through dehydrating-clearing schedule where the slides are dipped in eosin. Eosin is a very fast stain and therefore the sections should be kept for about one minute or less. Excess stain will be removed by adjusting the timings of treatment in 90 per cent and absolute alcohol. Sections are finally dehydrated in absolute alcohol, cleared in xylol and mounted in Canada balsam. If the dehydration is not perfect turbidity over the slide will appear when it is dipped in xylol or when mounted in Canada balsam medium which also contains xylol. The mounted slides are kept in a container away from dust. In a few days xylol evaporates and Canada balsam forms the solid layer over the sections. The advantage of using Canada balsam is that its refractive index is more or less same as that of glass and therefore distortion due to refraction is minimized when viewed under a microscope.

Causes of some common difficulties in sectioning: During microtomy often sections do not come properly due to one or more reasons which if identified correctly, can help correcting the defects. If all processes are carried out properly, good uniform undamaged sections can be obtained for microscopical examination. Some of the more common defects encountered during section-cutting are given below.

A. The sections fail to form a continuous ribbon.

(1) The paraffin is too hard.
(2) The microtome or the room is too cold.
(3) Sections are too thick.
(4) The blade is dull.

B. The ribbon is not straight, but curved.

(1) The lower edge of the block is not parallel with knife.
(2) The upper edge of the block is not properly trimmed; if the left side is too high the ribbon will curve to the right and *vice versa*.

C. The sections curl into a roll as these are cut.

(1) The paraffin is too hard.
(2) The blade edge is dull.
(3) The angle of the blade is not correct.

D. The sections compress and wrinkle as there are cut.

(1) The paraffin is too soft.
(2) The angle of the blade is incorrect.

E. There is a scrapping or ringing sound as the blade passes through the specimen or as the block returns to position on the upstroke.

(1) The specimen may be too hard to cut either because of the method of preparation or because of its natural characteristics.
(2) The angle of the blade is incorrect.
(3) The edge of the blade is too thin.

F. The sections vary markedly in thickness.

(1) The blade or the blade-holder is not held firmly in place.
(2) The specimen is not tightly clamped in the object clamp.
(3) The paraffin block is not firmly fixed.

G. Splits and tears appear in the sections.

(1) The edge of the blade is defective and has dents in it.
(2) There is dirt in the paraffin.
(3) There is dirt in or on the embedded specimen.

H. The material crumbles as it is cut or separates from paraffin matrix.

Some part of the dehydration, clearing or infiltration is at fault.

Constituents of reagnts used: A large number of fixatives, stains and other reagents are used in histological preparations. These are compounded by mixing various chemicals together. The ingredients of a few important reagents are given below.

Alcoholic grades

(i) From rectified spirit (96 per cent).

Required grade percentage	Rectified spirit in cc	Distilled water in cc
90	93.5	6.5
70	72.9	27.1
50	52.1	47.9
30	31.2	68.8

(ii) From 90 per cent alcohol.

Required grade percentage	90 per cent alcohol in cc	Distilled water in cc
70	77	23
50	55	45
30	33	67

Aqueous Bouin's fixative

Picric acid (saturated aqueous solution)	75 parts
Formalin (40 per cent)	25 parts
Acetic acid	5 parts

Alcoholic Bouin's fixative

95 per cent alcohol	250 parts
Water	750 parts
Picric acid	1 part

Carnoy's fixative

Absolute alcohol	6 parts
Chloroform	3 parts
Acetic acid (glacial)	1 part

Zenker's fixative

Potassium dichromate	2.5 gm
Mercuric chloride	5 gm
Distilled water	100 cc
Acetic acid (glacial)	5 cc

Mayer's albumen

White of egg	50 cc
Glycerine	50 cc
Sodium salicylate or thymol crystals	1 gm

Delafield's haematoxylin

Saturated solution of ammonia alum	400 cc
Haematoxylin crystals	4 gms dissolved in 25 cc alcohol

Mix above mentioned ingredients and leave it exposed to the light and air in an unstoppered bottle for three or four days. Filter and add 100 cc of glycerine and 100 cc of methyl alcohol. The solution should be kept for maturation or ripening until the colour is sufficiently dark, then filter. In case ripening process is to be shortened, the alum and haematoxylin solution should be exposed to the rays of copper-Hewitt burner in a shallow dish for an hour and then add the other ingredients and expose again the whole solution to light for two hours more.

Cleansing fluid for glassware

Potassium dichromate	20 gms
Distilled water	200 cc
Sulphuric acid	20 cc

Dissolve potassium dichromate in water, heat slightly if necessary; add slowly the acid to that and keep the solution in a stoppered bottle. It is highly corresive, hence handle with care.

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PAPER CHROMATOGRAPHY FOR AMINO ACID ANALYSIS

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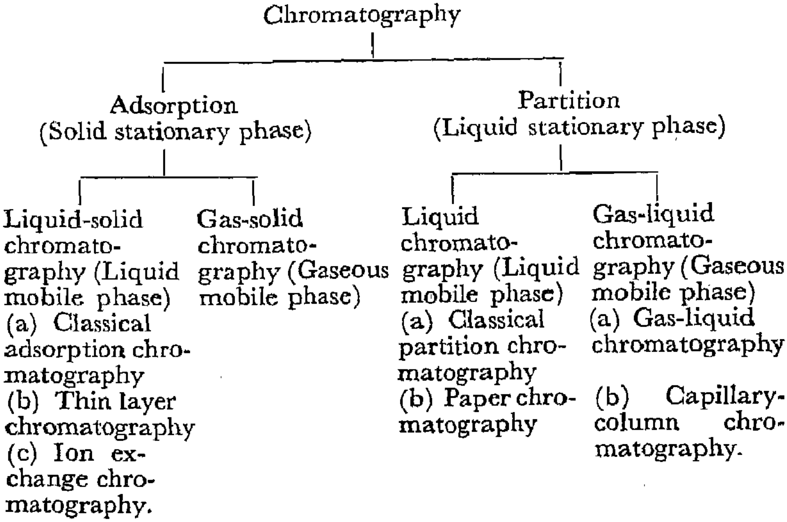
Chromatography is a physico-chemical technique for separating inorganic and organic constituents of a substance. The Polish botanist, Tswett is credited with the first successful application of chromatographic methods in the year 1906. This technique became popular with chemists and biologists as a result of the work done by Consden, Gorden and Martin in 1944 when they described in detail the method of paper chromatography. William and Kirby (1948) further improved this procedure. Today this technique is being used universally for separating proteins, amino acids, carbohydrates, steroids, antibiotics, vitamins and several other natural and synthetic substances. Unfortunately the word *chromatography* is a misnomer. Though Tswett showed a visible separation of coloured compounds, the essence of process of separation is not a colour partition of a solute but a partition of a solute between two solvent systems, usually liquids, one of them moving with respect to the other. Kinetic partition might be a better term, but still the word chromatography is commonly used. The feature common to all chromatographic methods is the use of two phases, one stationary and the other mobile. Separation depends on the relative movement between two phases.

Chromatographic methods may be classified according to nature of stationary phase which may be solid or liquid. If the stationary phase is solid the method is known as adsorption chromatography; if a liquid, as a partition chromatography. Since in each case the mobile phase may be either liquid or gas there are in all four types of chromatographic systems with their subtypes given below:

Paper Chromatography

Paper chromatography is cheap and simple and it has been used and adopted on a very wide scale. In this the stationary phase is the liquid layer supported by the cellulose particles of the filter paper. Mobile phase is also liquid. The rate of movement of a component of the mixture depends on its solubility in the stationary phase. More soluble substances travel


Chromatography
Adsorption
(Solid stationary phase)
Partition
(Liquid stationary phase)
Liquid-solid chromatography (Liquid mobile phase)
(a) Classical adsorption chromatography
(b) Thin layer chromatography
(c) Ion exchange chromatography.
Gas-solid chromatography (Gaseous mobile phase)
Liquid chromatography (Liquid mobile phase)
(a) Classical partition chromatography
(b) Paper chromatography
Gas-liquid chromatography (Gaseous mobile phase)
(a) Gas-liquid chromatography
(b) Capillary-column chromatography.

more slowly down the column than the less soluble. During the passage, the substances undergo partition between two phases and separation occurs depending upon the relative solubility of the substances in two different solvents.

Depending upon whether the solvent flows upwards or downwards the chromatography is of two types. In ascending method the paper is suspended so that its end from which flow starts is just below the surface of the solvent which ascends through the fibres of the paper by capillary action. In descending method the upper end of the paper is immersed in a suspended trough containing the solvent and the flow although started by capillary action, is continued by gravity.

Entomologists and other interested in insect biochemistry and nutrition have employed paper chromatography for the purpose of studying the amino acids of insects also as it is well known that insect blood contains very high concentration of free amino acids which is 20-100 times higher than that in human blood. Dent (1948) provided a map showing the positions of some sixty ninhydrin-reacting substances on phenol and collidine-lutidine (two dimensional) chromatograms for amino acid analysis.

General outline of the procedure: A drop of solution containing the mixture to be separated is placed on a marked position on a strip of filter paper where it spreads out to form a circular spot. When the spot has dried the paper is transferred to a container and the paper margin nearest to the spot is immersed into a solvent in such a manner that the spot is not in direct contact with the solvent because it will dissolve the spot off the paper. The solvent percolates through the fibres of the paper by capillary action and moves the components of the mixture to different extent in the direction of the flow. Small amount of water, held by adsorption on paper when in equilibrium with moist air, is sufficient to enable the chromatographic process to work. Those amino acids that are highly soluble in organic medium compared to their solubility in water will be carried along with the solvent while those that are relatively more soluble in water will remain behind. Under constant conditions, each amino acid moves a specific distance on the paper.

After the solvent has reached up to a predetermined level, the paper is removed from the container, the position of solvent front is marked and the strip is allowed to dry. Amino acids are colourless so these must be detected on the paper by physical or chemical means. The usual procedure is to spray with ninhydrin, a chemical that reacts with each amino acid to produce a readily observable coloured spot.

The colours given by most amino acids are purple grey but a few may give other colours *e.g.*, proline gives yellow, hydroxyproline pink, tyrosine and histidine blue grey. Pratt Jr. and Auclair (1948) have given a list of several amino compounds which give a visible colour with ninhydrin. The identification method is based on the position of the spot relative to solvent front using the R_f value.

$$R_f = \frac{\text{Distance moved by the substance}}{\text{Distance moved by the solvent front}}$$

The name R_f value was given by Consden, Gorden and Martin (1944). They found that R_f values were reproducible; that the R_f value of a particular amino acid varied according to solvent and since the relative values were not the same in all solvents; repeated experiments in a second solvent were sufficient to separate and characterize almost all common amino acids. It is therefore essential that the separation in chromatogram chamber must be carried at a constant temperature. It is customary to carry out the entire procedure in a laboratory fitted with air conditioners so that the results carried out at different times may be reproducible.

Sometimes, particularly in the case of large group of substances of similar chemical constitution such as amino acids, the R_f values are too close together to give a good separation whatever the solvent used. In such cases, it is necessary to use two different solvents in two dimensional chromatography This technique involves first separation with one solvent; the paper is dried and another solvent is run in other direction at right angle to the previous flow. The technique is described below in brief.

A full sheet of paper is used instead of a strip. The starting lines are marked along two sides at right angles about three inches from the edges. The starting point at which the mixture is applied will be the point of intersection of the two starting lines. The side near to the starting line is put into a solvent and allowed to drag the solute along in one direction. The paper is then removed and the solvent is dried off. The process is repeated in a second solvent in a direction at right angle to the first direction of flow. The chromatograph is dried and treated with the locating reagent. A pattern of spots will be formed. Identification here is usually done by comparison with 'standard map' obtained by chromatography of known individual amino acid and mixture.

Chromatograph chamber: Several types of air-tight chromatograph chambers are available commercially. The most widely used chamber is a rectangular vessel with a tightly fitting lid to prevent the escape of solvent vapours. At the top it has 3-6 tubular glass troughs which act as containers for the solvent. The margin of the paper with spots is held down by a glass rod inside the trough. To ensure that the solvents flow regularly and evenly the paper is passed over a glass rod-support, parallel to the edge of the trough. For first few centimetres, flow is by capillary action and gravitational flow begins only where the front has passed over the glass rod. The chamber should be thoroughly saturated with the solvent before running the chromatogram. For this, the solvent should be placed in the bottom of the chamber at least 24 hours before starting the run.

Types of paper: Numerous types of papers are recommended by various authors. Whatman No. 1 filter paper is most widely used. For separating larger quantities of solution sometimes thicker papers are used but these become too fragile.

Balston and Talbot (1952) recommended Nos. 4 and 5 for fast running; Nos. 1, 3 and 29 for medium running; Nos. 11 and 12 for medium slow running and Nos. 20 for slow running.

Sometimes copper is present as an impurity in the paper and shows as a pink 'ghost spot' near the true spot in some solvents.

Selection of solvents: Many solvent mixtures are used and Block *et al.*, (1952) described methods for preparing solvent mixtures. The choice of solvent depends on the nature of the substances to be separated. Good results have been obtained with the following solvent mixtures for separating amino acids.

(a) *Phenol-water*—To 75 grams of pure phenol, 25 ml of distilled water is added and warmed if necessary, to give colourless solution. A pink colour is sometimes obtained due to oxidation of phenol but it is not objectionable.

(b) *Butanol-acetic acid-water*—To 500 ml of freshly shaken mixture of equal volume of distilled water and *n*-butanol, is added 60 ml of glacial acetic acid in a separating funnel. After sometime the mixture separates into two layers. The lower layer is placed in the bottom of chamber for saturating it with water and solvent vapours. Upper layer is used as moving phase.

(c) *Collidine-lutidine-water*—This mixture is prepared by taking equal volumes of 2,4,6-collidine, 2,4 lutidine and water.

Standard map: Biological fluids usually contain 15 to 22 amino acids. A standard map of known amino acids is prepared before testing an unknown sample. R_f values of each amino acid is calculated for different solvents individually. A two-dimensional chromatogram of the amino acid mixture is run and spots are identified with the help of known R_f values.

The following 22 amino acids are made into solutions in distilled water for making standard map.

Amino acids	*mg/10 ml water*	*Amino acids*	*mg/10 ml water*
Alanine	50	Leucine	10
Arginine	60	Lysine	15
Asparagine	25	Methionine	50
Aspartic acid	10	Norleucine	10
Citrulline	25	Phenylalanine	25
Cystine	50	Proline	20
Glutamic acid	10	Serine	15
Glycine	10	Threonine	10
Histidine	50	Tryptophan	15
Hydroxyproline	10	Tyrosine	15
Isoleucine	10	Valine	15

It has been found that few amino acids are not readily soluble in water. In such cases a few drops of 10% HCl may be added.

Preparation of homogenate of insect tissue: Micks and Ellis (1952) described the methods as follows: 500 mg of starved insects are homogenized with 1.5 ml of water and centrifuged. From the supernatant, protein is precipitated by adding 0.7 ml of 95% ethanol and centrifuging it. To remove fats, supernatant is treated with 5 ml of chloroform and again centrifuged. The aqueous fraction is taken for amino acid analysis.

Spotting: The usual method in qualitative work is to touch the starting line with the tip of the glass capillary tube containing test solution and to allow the tip to remain in contact with the paper, until the capillary attraction by the paper has drawn enough liquid to form a round spot up to 1 cm in diameter. If the solution is very dilute it can be concentrated on the paper by applying a series of drops at the same place allowing each to dry completely before applying again. Hair drier may also be used for drying the spot quickly.

Identification of amino acid: The papers are thoroughly dried at room temperature and sprayed by 0.2% ninhydrin solution in 95% ethanol and again dried at 100°C for 5 minutes. R_f values of the spots are calculated and amino acids identified by comparing with the standard map of known amino acids. For quantitative analysis of amino acids, the ninhydrin-sprayed chromatogram is placed in a special reaction-cabinet containing carbon dioxide saturated with ethanol vapours at 60°C for 30 minutes. The colour spots are cut and eluted and the relative colour densities of the solutions are determined by a spectrophotometer. Another procedure, described by Block *et al.*, (1952) is based on the principle that the maximum colour density of each spot on a paper chromatogram is proportional to the concentration of the amino acid. The optical densities of the colour spots on the paper can be determined by means of photoelectric densitometer.

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INSTRUMENT FOR BIOLOGISTS

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The control of environmental conditions such as temperature, humidity *etc.*, has become essential in almost all the branches of scientific research. The control of any variable quantity is dictated by the deviation in the measured quantity with respect to a preset value. Therefore the accurate measurement of the quantity to be controlled is a basic part of the control system. There are various parameters which are controlled *viz.*, temperature, pressure, flow, humidity *etc.*

Temperature measurement and control

Let us discuss about the measurement of temperature as it is needed in our day to day work with ovens, incubators, water baths, refrigerators *etc.*

Temperature measurement

Temperature can be measured by different types of thermometers such as mechanical, fluid or electrical.

(1) *Metal expansion thermometers*—A linear rod of any metal expands when subjected to increasing temperature. The expansion in length is directly proportional to the temperature, therefore this principle can be used for temperature measurement.

(2) *Mercury in glass thermometers*—These are the simplest and most widely used temperature measuring elements and are suitable for temperature range from—38°F to +950°F. Because of their simplicity, these are also being used for clinical purposes.

(3) *Bimetallic thermometers*—These are widely used in industry and laboratory. When a junction of two different metals, one having low expansion and other high is subjected to temperature, a force is experienced. This force is proportional to the temperature difference. Usually for the low expansion metal Invar and for high expansion brass or nickle alloy are employed. This can be used for measuring the temperature ranging from 40°F to 800°F.

(4) *Liquid expansion thermometers*—Thermal expansion of a fluid is employed for the temperature measurement Toluene is widely used as it has very high coefficient of thermal expansion. Other details are same as mercury thermometers.

(5) *Pressure thermometers*—According to gas law $PV=RT$. The pressure of gas or vapour is proportional to its absolute temperature provided its volume is constant. If the pressure is measured by a Bourden tube or bellows, it would measure the temperature as well. The method is also employed for the measurement of gases and vapour in close containers.

(6) *Thermocouples*—A thermocouple is composed of two dissimilar metal wires welded together at one end. When hot junction is subjected to temperature the electron density of the two metals also increases. As these are different metals the electron density will be more in one than that in the other. This change in electron density develops an electromotive force or voltage which is linearly proportional to temperature. As this voltage is controlled by the temperature, it is known as thermo e.m.f. (electromotive force). A table of common combinations of thermocouple metals is given below :

Metal	Range °F	Microvolt per °F
+ Copper — Constantan	—300 to 600	33 mv
+ Iron — Constantan	0 to 1800	35 mv
+ Chromel — Alumel	600 to 1800	21.5 mv

As the voltage developed in this case is very small and weak in power, its measurement becomes difficult. Potentiometric and electronic methods are therefore employed to measure these voltages.

(7) *Resistance thermometers*—A resistance thermometer is widely used because of its recognised accuracy and simplicity. It is an electrical element, the electrical resistance of which varies with temperature. The measurement of electrical resistance gives the value of temperature of the surroundings in which the element is kept. A coil of fine wire such as platinum, nickel, or copper is wound on an insulating material like mica or glass. This resistance is usually measured by bridge circuits.

(8) *Thermistors*—This is another temperature measuring device and is similar to a resistance thermometer. Thermistors are made from mixtures of metal oxides and provide a resistance versus temperature relation. The resistance may either decrease or increase with the rise in temperature. When the resistance increases with temperature it is known as positive coefficient-type thermistor and when decreases with the temperature as negative coefficient-type thermistors.

Temperature control : In order to obtain a definite constant temperature in an oven or incubator, a person can be deputed who can see the thermometer and can switch on and off the heater accordingly, when the temperature rises and falls below the required range. This type of manual control is costly, as well as it involves the human errors. Very cheap control units are now available, which can replace the manual control and can give accurate and error free service. These automatic controls can be employed in various modes; the simplest of them is a two position or on-off control. The same is usually employed in most of the ovens, refrigerators, incubators and waterbaths.

The electrical operation of a heater or cooler is controlled by make and break circuit which is governed by a relay. The relay is operated by the thermometric operation. A mercury thermometer can be used as a relay (Fig. 33-1) if a thin wire is employed as the electrical circuit element which makes the circuit on when mercury touches this wire and otherwise disconnects the circuit. Bimetallic relays are most widely used 'sensing and controlling elements'. These are available both in as 'normally on' and 'normally off' modes to suit the requirements of heating as well as cooling. The mercury thermometers require additional relay to operate the heating and cooling devices because only a very small current can pass through the mercury circuit. In order to pass heavy currents as in heaters and coolers additional relays of high current capacity are employed. Bimetallic relays are available in high current ratings too, therefore these do not require additional relays (Fig. 33-2). In the above two temperature control circuits, indicator lamps are shown in series with the heater in order to make the circuit simpler. However these indicator lamps are generally connected in parallel with the heater.

Colourimetry and spectrophotometry

The discovery of photoelectric effect has made possible the accurate measurement of intensity of light. As evident from its name, the phenomenon of photoelectric effect states that when light rays (photons) fall on a photosensitive surface, electrons are liberated from that surface. The number of electrons emitted out will be proportional to the number of photons of the incident radiation. These electrons can be collected by a positive electrode with respect to the photo surface when both are placed in a vacuum tube (Fig. 33-3). The current which depends upon the number of electrons and the voltage of anode gives a linear relationship with the intensity of light.

The above type of photoelectric device is known as *photoemissive cell.* These are available in vacuum cell types as well as gas filled types. It is to be noted that a stabilized power supply is needed to provide anode voltage.

The second type of photo-cells are known as photovoltaic cells. These cells are made of selenium metal surrounded by two metallic thin plates which are also used as electrodes. When light rays fall on this photo sensitive surface, voltage is developed across the two electrodes. This voltage is proportional to the intensity of light. Hence, if this voltage can be measured accurately the readings will provide a measure

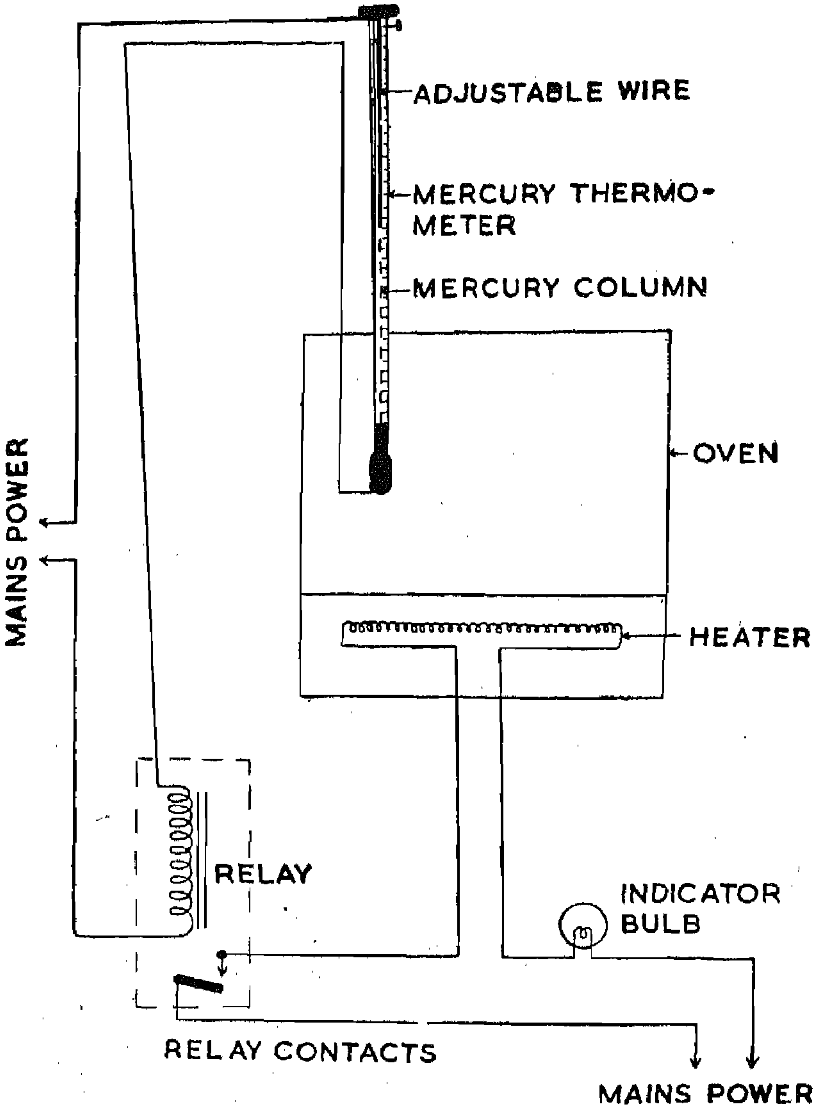

Fig. 33-1. Mercury thermometer type thermostat with relay.

of light intensity. These photovoltaic cells are widely used in colourimeters, exposure meters *etc.*

The third type of photocells are known as photoconductive cells. The conductivity or resistance of this cell varies with the amount of light incident on it. The change of resistance or conductivity will be a function of the intensity of light. Since the relation is not very linear and because of its

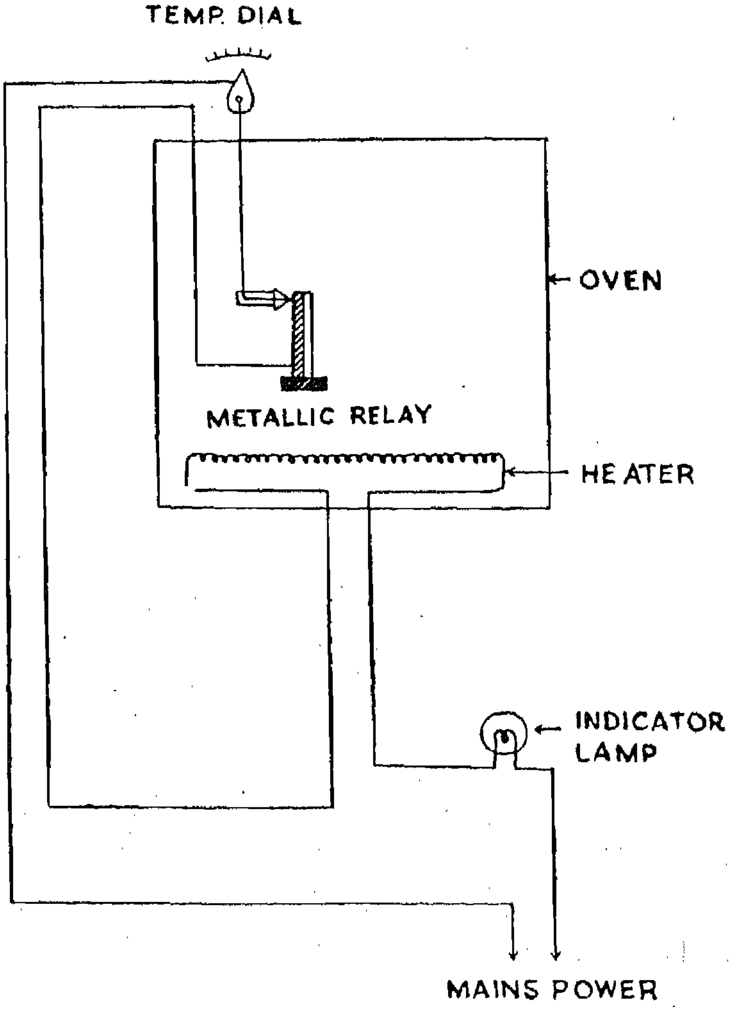

Fig. 33.2 Bimetellic thermostatic control

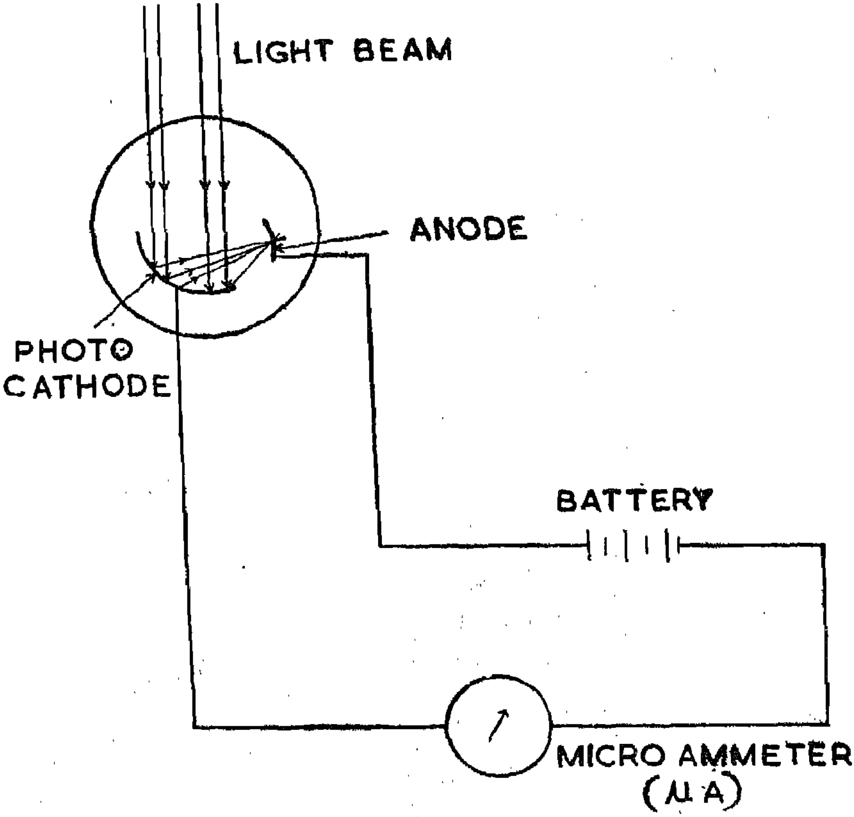

Fig. 33-3. A Photocell with circuit for measurement of intensity of light

conductivity even in the dark, this type of photocells are very rarely used.

Colourimeters : Colourimeters or photometers are used for the measurement of optical density of coloured solutions. Various substances give a specific colour when these are dissolved in some solvent, and the optical density of this solution has a linear relation with the concentration. Optical density and transmission are also related to each other. What we measure by photometers is the percentage of light which is transmitted through the solution. It is mostly taken with reference to 100 per cent transmissions of distilled water.

In simplest type of photometer (Fig. 33-4) the light from a lamp is subjected to fall on the photocell through a filter and the soultion under investigation. The photocell is connected to a sensitive spot-galvanometer which measures the voltage across

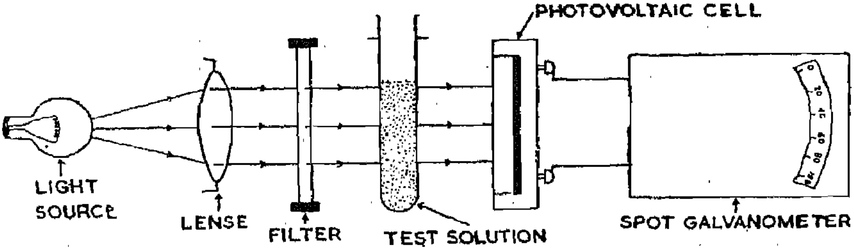

Fig. 33-4. A simple type of colourimeter with one photocell.

the photocell. In this type of colourimeter, the intensity of light bulb should be kept constant otherwise fluctuations in this may cause errors in meter readings.

In order to compensate this error, a double beam photometer is generally employed. One beam falls on the photocell through the sample, while other falls on a reference photocell. The difference of two is measured by a galvanometer. The fluctuations in bulb light intensity will produce changes in both photocells and therefore the difference will be. neutralized.

Spectrophotometers: As discussed earlier, in a colourimeter coloured glass filter is used in order to pass a definite portion of the wave length spectrum of light. This wave length range of the filter is too large for the advanced methods of analysis which requires higher resolutions involving the absorption by liquid only at some particular wave lengths within ±5A° or so. A prism or a grating can be employed to produce a sharp spectrum. The rotation of prism or grating will allow a particular wave length range of spectrum to fall on the slit of the spectrophotometer. The light passes through this slit to the solution under test and then to the photocell. In this case the light after splitting in spectrum becomes very weak in intensity and its measurement by photovoltaic cells becomes difficult. Sensitive photoemissive cells are generally employed for this purpose. The photocell output which used to be very weak is amplified and displayed on a meter.

pH measurement

The pH is a simple number for expressing the degree of effective acidity or alkalinity of a solution. Mathematically it is the negative logarithm of the hydrogen ion concentration, $pH = -\log(H^+)$

It can be seen that the pH scale is inverted *i.e.*, lower the pH greater the hydrogen ion concentration. For all practical purposes the pH of aqueous solutions lies between 0 to 14.

From 0 to 7 it is in acidic range and from 7 to 14 alkaline range. The pH 7 is neutral.

Measurement of pH by electrical methods: Although there are colour indicators for pH estimations but their accuracy is limited by the judgement of human eye. After the development of colourimeters and spectrophotometers it has become possible to determine the pH by this method. The usual electronic pH meters employ the development of voltage on a hydrogen ion sensitive electrode (such as glass electrode) with respect to a reference electrode (calomal electrode), when both of these electrodes are dipped in the solution under test. The pH of the solution is given by the difference of voltages on the two electrodes.

$$\text{pH} = \frac{\text{Eg—Eref.}}{0.0591} \text{ at } 25°\text{C}$$

Hence the measurement of this voltage which is of the order of few millivolts, provides the measurement of pH.

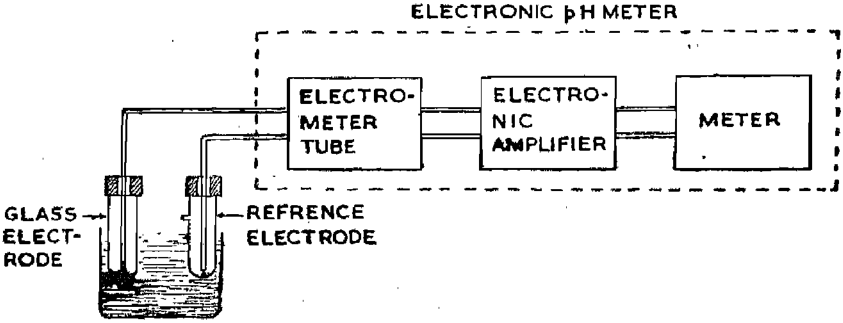

Fig. 33-5. Electronic direct reading pH meter.

Since this voltage is very weak in power, it cannot operate a needle type voltmeter. Special electrometer tubes are therefore used for the measurement of this voltage. These electrometer tubes consume very little current (10^{-8} amp) from the source under test, without affecting the voltage. The output of the electrometer tube is amplified and recorded on a needle type meter (Fig. 33-5).

Operation of pH metres: Turn the instrument on and allow warm up period of 15 minutes. Place about 25 ml of buffer of known pH in a 50 ml beaker and immerse both the electrodes. Set the temperature knob at the temperature of the buffer. If the pH meter has two ranges 0-7 and 7-14, then switch to the proper range. Adjust the meter needle to the pH of the buffer by standardization knob. Wash the electrodes with distilled water and then rinse with the test solution.

Place about 25 ml of test solution in a beaker and immerse the electrodes. Set the temperature knob at the temperature of the test solution and read pH value.

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ELECTRON MICROSCOPY IN THE STUDY OF BIOLOGICAL SCIENCES

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THE ultimate aim of biologists and biochemists is to study all biological processes at the atomic and molecular levels. To see atoms and molecules (1-100A°) X-ray diffraction as well as small-angle X-ray and electron diffraction are used. All these methods give indirect information about the structure. To study large molecules (50-1000A°), and supra-molecules (1000-10000A°) and sub-cellular parts (10000 and above) only electron microscopy is used. Electron microscopy can also give direct information up to 10A° molecules. This information is extremely useful to study the biological functions wih regard to molecular structure. Electron microscope has been put to excessive use to study macro-molecular structure of filamentous proteins like collagens and membranous structures formed by lipo-protein complexes. The sites of iron accumulation within ferritin molecules (mol wt of protein portion=4,65,000) were also seen by electron microscope. With negative staining, subdivision of filamentous structures can be seen. To have the idea of how to study these biological systems we should know briefly the principles of electron microscopes and the various techniques employed.

Basic principles

To study any object we should have some means by which we can get information about the object to be seen. Thus any radiation which is used to observe the object, interacts with the object and carries the information about the structure. At the atomic level the radiation behaves in wave form. This inherent property of radiation depends upon its momentum and the nature of its interaction with matter. Thus the wave length of light quantum is of the order of a few thousand A° but that of electron is only a fraction of an angstrom. Due to this wave nature of the radiation, if we want to image some point of the object we can never get a sharp point image but a broad bright point with bright light rings around it *i.e.*, we get the diffraction pattern of the point. The intensity in the first bright point or first maxima is 89% of the total scattered intensity by the object. The rest of the intensity is distributed in successive maximas (rings). In case of a microscope if the point is observed by, say light radiation and is imaged by the objective lens then the image of the point will be the diffraction pattern of that point by the aperture of the objective lens. For all practical purposes the central maxima is taken as the image of the object and this is called the *airy's disc.*

The resolving power *i.e.*, the minimum distance between two points which can be clearly seen is defined according to Rayleigh as that distance between two maximas of diffraction if the centre of airy's disc of one point falls on the circumference of the other. Using this criterion, resolving power is equal to 0.61 λ/α where λ is the wave length of the radiation used and α is the numerical aperture of the objective lens which controls the amount of the radiation from the object to be imaged. In electron microscope the radiation used is electron beam accelerated to a high potential and the numerical aperture is 1 because the object is kept under vacuum and in the focal plane of objective lens.

Electron microscope

In electron microscope we use highly accelerated electron beams emitted from a point source. The acceleration in conventional microscopes is by a potential of the order of few hundred kilo volts. To give maximum resolving power the diameter of the beam is made very small to have a coherent source *i.e.*, the source in which the same phase relationship exists between two nearby points. This source is used to illuminate the object. The diameter of beam is made very small by using double condenser lens system. The first lens focuses large number of electrons into a small spot. This gives sufficient electron density to give illumination at higher magnification. Second lens focuses this spot on the specimen. The lenses used for illuminating-system as well as the remaining lenses *i.e.*, objective lens and the intermediate lens-assembly can be of electrostatic or electromagnetic type. In the electrostatic type, potential difference between two co-axial conducting cylinders is used for the lens action. In the electromagnetic type, magnetic field generated by a coil in the form of a solenoid is used to focus electrons. The shape of the field is controlled by different shapes of the pole shoes. The final image is observed on a fluorescent screen. Whole of the electron microscopic column is evacuated to a high vacuum to avoid absorption and scattering of electrons by the gas molecules. Small apertures are used in all the lenses to avoid spherical aberrations and to get contrast in the image. Special aberrations adversely affect the resolving power of the microscope. Another electron optical defect which affects the resolving power is the astigmatism of two principal lenses *i.e.*, the condenser and the objective lens. This defect is generated due to asymmetry of the fields and is corrected by compensating fields. The object to be studied should be thin enough to avoid absorption and incoherent scattering of the electrons.

Theory of image formation

In optical microscope the image is primarily formed by the selective absorption of light by the object. But in electron microscope the absorption of electrons in the specimen is negligible due to high energy of the electrons and the basic process for image formation is scattering and diffraction. Thus there are four main processes in the image formation *viz.*, (i) scattering, (ii) diffraction effects of the object, (iii) refraction and phase contrast and (iv) diffraction by electron-optical systems.

Scattering: Scattering of the electrons depends upon the charge on the nucleus of the atoms of the object and repulsion of electrons by the electrostatic interaction with the nuclei and core-electrons of the atoms. The collisions can be elastic or inelastic. In the elastic collisions the energy of the electron is not lost but in the inelastic collision energy is lost and thus the electrons are deflected more and add to the contrast of the object. The probability of this collision depends upon the atomic number of the scattering atoms in the object. Probability of scattering also depends upon the density of electrons in the object. Thus in crystalline solids the contrast is more.

Diffraction effects of the object: Fresnel type of diffraction pattern of opaque objects are formed at the objective aperture of the electron microscope. The prerequisite for the formation of diffraction pattern is the coherence of the electron beam, *i.e.,* the phase difference should remain constant with time. In opaque and semi-transparent objects under under-focused condition of the objective lens, anomalous scattering takes place. This phenomenon gives the diffraction contrast in the image. The anomalous scattering takes places probably due to surface charges on the objects. This point should be remembered in the interpretation of laminar and granular fine structures of objects due to the above contrast effect. Under this effect, laminated single-membrane structure appears as multilayered structure, hence to observe such structures exactly focused patterns should be taken at the expense of contrast to some extent in the image. To observe fine particles, under-focused pattern should be taken for better contrast thereby adding to the resolution.

Refraction and phase contrast: If the object is fully transparent to the electron beam as in ultra thin sections but has different regions of refractive indices, phase difference can be produced in transmitted beam, and if this phase difference is quarter of the wave length of the electrons, destructive interference can occur, thus producing phase contrast. Quarter phase difference is introduced in the electron microscope in the diffracted beam from the object, not by a quarter wave plate as in an optical microscope but by slightly defocusing the objective lens. Defocusing changes the optical pattern for the scattered electron as compared to the non-scattered one due to the spherical aberration at the objective aperture thus increasing the path difference of the scattered electrons. This produces conditions for destructive interference producing phase contrast.

In the matrix of thin sections, regions of different electric charges are present which change the electrical potential inside the object. This causes refraction effect of the traversing electrons introducing a phase difference compared to the unrefracted electrons and causes phase contrast due to refraction. Positive and negative charges give reverse effects.

Diffraction by the electron-optical systems: In the electron optical system well contrast images are formed in the following manners :

(i) *Contrast due to Bragg's reflection*—In certain objects micro-crystalline and semi-crystalline states exist. These states give conditions of Bragg's reflection giving thereby sharp contrast in their images.

(ii) *Dark field images*—These images are produced from crystalline to semi-crystalline object by allowing only the scattered electrons from the object to form the image. Direct electron beam is avoided either by shifting the object slightly away from the electron optical axis of the microscope or by tilting the electron beam on the object to a small angle with the electron optical axis of the microscope. This contrast due to scattered electrons gives those crystalline features of the objects which are not visible by direct microscopy.

Techniques for specimen preparation for electron microscope

Preparation of support films: For the study of biological object under electron microscope the material is supported by thin supporting films which can be prepared from plastics-like collodion or formvar and from inorganic amorphous substances like carbon or silicon monoxide. Plastic films though less brittle are denatured under electron beam and hence are not used for high resolution work. For high resolution work carbon or silicon monoxide films are used which are brittle to some extent. To avoid defects due to the brittleness, backed carbon films are used. Backing is done by a very thin film of collodion or bedacryal. Plastic films are prepared from very dilute solutions of the above mentioned plastics in some organic solvents which are spread over the surface of water in a trough. The thickness of the film is controlled by dilution. Carbon and silicon monoxide films are prepared by evaporating these materials under vacuum on a clean glass substrate. The film is peeled off from the glass substrate on the water surface. Copper grids are mounted on both types of films to form support-films for mounting specimens for electron microscopy.

Studies of biological objects: The study of biological objects falls into three main categories as described below. This comprises study of small particles like viruses and bacteriophage and large particles like bacteria, microtubules, mitochondrial fragments *etc.*

Particulate studies—All these particles can be studied by negative staining. Larger particles can also be studied by shadow casting. Negative staining is done by using a concentration of about 10^7-10^8 particles per cc. The preparation is mounted on the support films and after drying it, a two per cent solution of potassium phosphotungstate at pH 6.7 in ammonium acetate buffer is used to stain the particles for a very short duration. Uranyl formate (0.5 to 1%) at pH 4.0-5.2 is best for staining under aqueous conditions. For spreading the stains, 0.005 to 0.05% glycerine or propylene glycol is added in the particle preparation. The mixture of particle-preparation and stain on the prepared grids should never be stored because the particle structure rapidly breaks down under these conditions.

Surface studies—Surface studies of the biological samples (bacteria, pollen grains, leaves, fungal spore *etc.*,) can be done by preparing the replicas of their surfaces. As the surfaces of membranes and organalles inside the cell cannot be studied by thin sectioning method, the freeze etching technique is used for preparing surface replicas of such objects. Surface replicas can be prepared in two ways *viz.*, single stage replicas and multistage replicas. In the single stage replica, reverse impression comes whereas in the multistage replicas, the direct impression is studied. Replica of the surface can be formed either by a plastic film over the surface or by carbon evaporation in vacuum. Plastic films are peeled off directly from the surface and are shadow-casted before examination. In the preparation of carbon replicas the first impression is usually taken on a plastic film over which carbon film is evaporated and the plastic film is later dissolved in an organic solvent leaving behind the replica which is shadow-casted and observed in the electron microscope. Carbon replicas can also be prepared by directly evaporating carbon on the objects like spores, pollen grains and microorganisms. The carbon film is separated from the substrate and the objects are removed by oxidising in a suitable reagent. Replicas are then washed and shadow-casted and observed.

In freeze etching technique, the object is quickly frozen in a cold bath of liquid nitrogen under high vacuum to avoid ice crystal formation over the object. The object is then fractured slowly with a razor blade moved by a micro-lathe. The fractured surface which should be shining, is allowed to sublime with water vapour. Over this sublimated surface a self-shadowed carbon replica under vacuum is prepared. The replica with the object is slowly raised to the normal temperature and later on the temperature is slightly raised out side the vacuum to detach the replica from the object by melting the ice. Some substances which are harder to freeze because of high water content are added in a 20% glycerol solution to be penetrated inside it. Here glycerol acts as an antifreeze agent. Some workers prefer to prefix the tissue in 2% gluteraldehyde at room temperature for a suitable time before treating with 20% glycerol solution. This technique is extremely delicate and requires a highly skilled worker to do it.

Shadow-casting of the prepared replica or the particles to be studied is done by evaporating a heavy metal or an alloy under vacuum over the specimen-mounted grids at an oblique angle. For studying very fine features shadowing at a very low angle is preferred.

Ultra thin section studies: For cytochemical and genetic studies very thin sections (200-800A°) of the specimen are prepared through the same stages as those in the optical microscopy. The only difference is that the ingredients used in all these stages are different and the physio-chemical conditions are to be accurately adjusted. Thus samples are first fixed, then washed and dehydrated. After the dehydration these are embedded in certain monomers of plastics and are later polymerized in the form of blocks which are cut into very thin sections with ultramicrotomes using glass or a diamond knife. Since the sections are very thin these are floated on water surface for collection. The thickness of the sections which is controlled by a mechanical or thermal feed, is judged by the colour of interference from the thin sections. Silver grey (200-300A°) are the best sections for electron microscopy. Sections are later picked up on the support-film mounted on grids and stained before observation. In some specimens prestaining at the stage of dehydration is preferred. Double staining is done for differential studies of the cellular components.

Several types of fixatives are used in electron microscopy depending upon the nature of the material and type of studies to be made. Aldehyde fixative like formaldehyde and gluteraldehyde penetrate well, to preserve fine structures of the cell. These fixatives are less drastic in their chemical action. Fixatives like osmium tetraoxide and potassium permanganate are used for fixing membranes, cell walls and other large and hard structures. Fixative action is influenced by the pH, temperature and the duration of treatment. These parameters should be critically adjusted for specific specimens. Double fixation is always preferred for a better structural fixation and generally the first is an aldehyde fixative to fix the delicate structure followed by osmium tetraoxide or potassium permanganate. These fixatives of proper concentration are prepared in suitable buffer which controls the pH and tonicity of the tissue. The buffers used are veranol acetate, phosphate and sodium cacodylate. The fixed tissues are washed in buffers and dehydrated in alcohol, acetone or propylene oxide. The infiltrating resins can be divided into three categories, *viz.*, (i) long chain plastics, like methacrylate, (ii) crossed link plastics like araldite Spon, Vestopol-W and ERL-4206 and (iii) water soluble resins like aquon, glycol-methacrylate and duracupan. First category of the plastics suffer polymerization damage, hence in the final stage of infiltration, a treatment with prepolymerized resins is given to minimise the damage. These plastics are also not stable under the electron beam and undergo sublimation hence are not used for high resolution work. Second category plastics are the best so far known to preserve the biological structures. The third category plastics in which aquon is the best, are used for cytological and enzymic studies where post chemical treatments in the thin sections can be given. As these are hygroscopic the sectioned grids should be stored in vacuum.

Common stains used in electron microscopy are (i) uranyl acetate, (ii) potassium permanganate, (iii) lead compounds like lead citrate and lead hydroxide. In double staining usually uranyl acetate is followed by lead or potassium permanganate stain.

Generally all the above chemical treatments for the study of these sections alter the micro-structure to some extent. To avoid this, sectioning of the tissues is done without any chemical treatment. This is done by rapidly freeze-drying the sample, thus preserving the structure to the greatest extent and sectioning the tissue at low temperatures. Sections are picked up on the grids and studied directly.

Some recent advancements in techniques: Recently techniques for directly seeing the surfaces and organalle structures in the cell have been developed. This is done by scanning electron microscope. In this, though a high resolution has not yet been achieved, one can see the three dimensional information about the specimens. Prepared specimens are treated with some metallic coating which is given a negative-biased voltage. The voltage distribution depends upon the constitution as well as the topography of the specimen. An electron beam scans the specimen like a TV camera. The secondary emission of electrons from the specimens, either in the transmission or in the reflection mode is synchronized and amplified to give the final image.

These days attempts are also being made to see the biological processes in living cells. A step towards this goal has been taken by using a very high energy electron beam (1-2 million e.v. at present) for imaging. Due to the high energy of the beam, the electrons pass through the cells without causing much damage as was earlier feared. Thus these can penetrate quite thick specimen chambers where the living conditions of the cells are maintained. Further work on these aspects is being carried out at various places in the world.

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* Names wherever given in parantheses are the accepted ones at present, though referred in the book by different names.